

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**



PATENT
674523-2030

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s) : KINGSMAN, et al.
Serial No. : 10/690,320
For : VECTOR SYSTEM
Filed : October 20, 2003
Art Unit : To Be Assigned

745 Fifth Avenue, New York, NY 10151

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on December 15, 2003.

Anne-Marie C. Yvon, Reg. No. 52,390

(Name of Applicant, Assignee or Registered Representative)

Anne-Marie C. Yvon
Signature

December 15, 2003

Date of Signature

COMMUNICATION

Commissioner for Patents
P.O. Box 3 1450
Alexandria, VA 22313-1450

Sir:

Enclosed are certified copies of priority documents for the above named application.

Applicants hereby claim priority under 35 U.S.C. §§119 and 120 from International Patent Application No. PCT/GB02/01830 and United Kingdom Patent Application No. GB0109781.5

Acknowledgment of the claim of priority and of the receipt of said certified copies are respectfully requested.

Respectfully submitted,
FROMMER LAWRENCE & HAUG LLP

By: Anne-Marie C. Yvon
Thomas J. Kowalski, Esq.
Reg. No. 32,147

Anne-Marie C. Yvon, Ph.D.
Reg. No. 52,390
T:(212) 588-0800



INVESTOR IN PEOPLE

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation and Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents held on the international application filed on **19th April 2002** under the Patent Cooperation Treaty at the UK Receiving Office. The application was allocated the number **PCT/GB2002/001830**.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or the inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed

Date:

31 October 2003

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No. **PCT/GB 02/01830**

International Filing I **19 APRIL 2002**

Name of receiving Office **United Kingdom Patent Office**
PCT International Application

Applicant's or agent's file reference
(if desired) (12 characters maximum) **P011367WO CLM**

Box No. I TITLE OF INVENTION VECTOR SYSTEM	
Box No. II APPLICANT <input type="checkbox"/> This person is also inventor	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)	
OXFORD BIOMEDICA (UK) LIMITED Medawar Centre Robert Robinson Avenue The Oxford Science Park Oxford OX4 4GA GB	Telephone No. Facsimile No. Teleprinter No. Applicant's registration No. with the Office
State (that is, country) of nationality: GB	State (that is, country) of residence: GB
This person is applicant for the purposes of: <input type="checkbox"/> all designated States <input checked="" type="checkbox"/> all designated States except the United States of America <input type="checkbox"/> the United States of America only <input type="checkbox"/> the States indicated in the Supplemental Box	
Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)	
KINGSMAN, Susan A Graystones <i>Medawar Centre</i> Middle Street Islip <i>Robert Robinson Avenue</i> Oxfordshire OX5 2SF <i>The Oxford Science Park</i> GB <i>Oxford OX4 4GA</i>	This person is: <input type="checkbox"/> applicant only <input checked="" type="checkbox"/> applicant and inventor <input type="checkbox"/> inventor only (If this check-box is marked, do not fill in below.) Applicant's registration No. with the Office
State (that is, country) of nationality: GB	State (that is, country) of residence: GB
This person is applicant for the purposes of: <input type="checkbox"/> all designated States <input type="checkbox"/> all designated States except the United States of America <input checked="" type="checkbox"/> the United States of America only <input type="checkbox"/> the States indicated in the Supplemental Box	
<input checked="" type="checkbox"/> Further applicants and/or (further) inventors are indicated on a continuation sheet.	
Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE	
The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: <input checked="" type="checkbox"/> agent <input type="checkbox"/> common representative	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)	
MALLALIEU, Catherine Louise D Young & Co 21 New Fetter Lane London EC4A 1DA ENGLAND	Telephone No. +44 20 7353 4343 Facsimile No. +44 20 7353 7777 Teleprinter No. 477667 YOUNGS G Agent's registration No. with the Office
<input type="checkbox"/> Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.	

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)	
<i>If none of the following sub-boxes is used, this sheet should not be included in the request.</i>	
<p>Name and address: <i>(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)</i></p> <p>MITROPHANOUS, Kyriacos c/o Oxford BioMedica (UK) Limited Medawar Centre Robert Robinson Avenue The Oxford Science Park Oxford OX4 4GA GB</p>	<p>This person is:</p> <p><input type="checkbox"/> applicant only</p> <p><input checked="" type="checkbox"/> applicant and inventor</p> <p><input type="checkbox"/> inventor only <i>(If this check-box is marked, do not fill in below.)</i></p> <p>Applicant's registration No. with the Office</p>
State <i>(that is, country)</i> of nationality: GR	State <i>(that is, country)</i> of residence: GB
<p>This person is applicant for the purposes of:</p> <p><input type="checkbox"/> all designated States <input type="checkbox"/> all designated States except the United States of America <input checked="" type="checkbox"/> the United States of America only <input type="checkbox"/> the States indicated in the Supplemental Box</p>	
<p>Name and address: <i>(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)</i></p> <p>ELLARD, Fiona Margaret c/o Oxford BioMedica (UK) Limited Medawar Centre Robert Robinson Avenue The Oxford Science Park Oxford OX4 4GA GB</p>	<p>This person is:</p> <p><input type="checkbox"/> applicant only</p> <p><input checked="" type="checkbox"/> applicant and inventor</p> <p><input type="checkbox"/> inventor only <i>(If this check-box is marked, do not fill in below.)</i></p> <p>Applicant's registration No. with the Office</p>
State <i>(that is, country)</i> of nationality: GB	State <i>(that is, country)</i> of residence: GB
<p>This person is applicant for the purposes of:</p> <p><input type="checkbox"/> all designated States <input type="checkbox"/> all designated States except the United States of America <input checked="" type="checkbox"/> the United States of America only <input type="checkbox"/> the States indicated in the Supplemental Box</p>	
<p>Name and address: <i>(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)</i></p>	<p>This person is:</p> <p><input type="checkbox"/> applicant only</p> <p><input type="checkbox"/> applicant and inventor</p> <p><input type="checkbox"/> inventor only <i>(If this check-box is marked, do not fill in below.)</i></p> <p>Applicant's registration No. with the Office</p>
State <i>(that is, country)</i> of nationality:	State <i>(that is, country)</i> of residence:
<p>This person is applicant for the purposes of:</p> <p><input type="checkbox"/> all designated States <input type="checkbox"/> all designated States except the United States of America <input type="checkbox"/> the United States of America only <input type="checkbox"/> the States indicated in the Supplemental Box</p>	
<p>Name and address: <i>(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)</i></p>	<p>This person is:</p> <p><input type="checkbox"/> applicant only</p> <p><input type="checkbox"/> applicant and inventor</p> <p><input type="checkbox"/> inventor only <i>(If this check-box is marked, do not fill in below.)</i></p> <p>Applicant's registration No. with the Office</p>
State <i>(that is, country)</i> of nationality:	State <i>(that is, country)</i> of residence:
<p>This person is applicant for the purposes of:</p> <p><input type="checkbox"/> all designated States <input type="checkbox"/> all designated States except the United States of America <input type="checkbox"/> the United States of America only <input type="checkbox"/> the States indicated in the Supplemental Box</p>	
<p><input type="checkbox"/> Further applicants and/or (further) inventors are indicated on another continuation sheet.</p>	

Box No. V DESIGNATION OF STATES

Mark the applicable check-boxes below; at least one must be marked.

The following designations are hereby made under Rule 4.9(a):

Regional Patent

- ☒ **AP ARIPO Patent:** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, MZ Mozambique, SD Sudan, SL Sierra Leone, SZ Swaziland, TZ United Republic of Tanzania, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- ☒ **EA Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ☒ **EP European Patent:** AT Austria, BE Belgium, CH & LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, TR Turkey, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ☒ **OA OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT (if other kind of protection or treatment desired, specify on dotted line)

National Patent (if other kind of protection or treatment desired, specify on dotted line):

- | | | |
|---|--|--|
| <input checked="" type="checkbox"/> AE United Arab Emirates | <input checked="" type="checkbox"/> GH Ghana | <input checked="" type="checkbox"/> MX Mexico |
| <input checked="" type="checkbox"/> AG Antigua and Barbuda | <input checked="" type="checkbox"/> GM Gambia | <input checked="" type="checkbox"/> MZ Mozambique |
| <input checked="" type="checkbox"/> AL Albania | <input checked="" type="checkbox"/> HR Croatia | <input checked="" type="checkbox"/> NO Norway |
| <input checked="" type="checkbox"/> AM Armenia | <input checked="" type="checkbox"/> HU Hungary | <input checked="" type="checkbox"/> NZ New Zealand |
| <input checked="" type="checkbox"/> AT Austria | <input checked="" type="checkbox"/> ID Indonesia | <input checked="" type="checkbox"/> PL Poland |
| <input checked="" type="checkbox"/> AU Australia | <input checked="" type="checkbox"/> IL Israel | <input checked="" type="checkbox"/> PT Portugal |
| <input checked="" type="checkbox"/> AZ Azerbaijan | <input checked="" type="checkbox"/> IN India | <input checked="" type="checkbox"/> RO Romania |
| <input checked="" type="checkbox"/> BA Bosnia and Herzegovina | <input checked="" type="checkbox"/> IS Iceland | <input checked="" type="checkbox"/> RU Russian Federation |
| <input checked="" type="checkbox"/> BB Barbados | <input checked="" type="checkbox"/> JP Japan | |
| <input checked="" type="checkbox"/> BG Bulgaria | <input checked="" type="checkbox"/> KE Kenya | <input checked="" type="checkbox"/> SD Sudan |
| <input checked="" type="checkbox"/> BR Brazil | <input checked="" type="checkbox"/> KG Kyrgyzstan | <input checked="" type="checkbox"/> SE Sweden |
| <input checked="" type="checkbox"/> BY Belarus | <input checked="" type="checkbox"/> KP Democratic People's Republic of Korea | <input checked="" type="checkbox"/> SG Singapore |
| <input checked="" type="checkbox"/> BZ Belize | <input checked="" type="checkbox"/> KR Republic of Korea | <input checked="" type="checkbox"/> SI Slovenia |
| <input checked="" type="checkbox"/> CA Canada | <input checked="" type="checkbox"/> KZ Kazakhstan | <input checked="" type="checkbox"/> SK Slovakia |
| <input checked="" type="checkbox"/> CH & LI Switzerland and Liechtenstein | <input checked="" type="checkbox"/> LC Saint Lucia | <input checked="" type="checkbox"/> SL Sierra Leone |
| <input checked="" type="checkbox"/> CN China | <input checked="" type="checkbox"/> LK Sri Lanka | <input checked="" type="checkbox"/> TJ Tajikistan |
| <input checked="" type="checkbox"/> CO Colombia | <input checked="" type="checkbox"/> LR Liberia | <input checked="" type="checkbox"/> TM Turkmenistan |
| <input checked="" type="checkbox"/> CR Costa Rica | <input checked="" type="checkbox"/> LS Lesotho | <input checked="" type="checkbox"/> TR Turkey |
| <input checked="" type="checkbox"/> CU Cuba | <input checked="" type="checkbox"/> LT Lithuania | <input checked="" type="checkbox"/> TT Trinidad and Tobago |
| <input checked="" type="checkbox"/> CZ Czech Republic | <input checked="" type="checkbox"/> LU Luxembourg | <input checked="" type="checkbox"/> TZ United Republic of Tanzania |
| <input checked="" type="checkbox"/> DE Germany | <input checked="" type="checkbox"/> LV Latvia | <input checked="" type="checkbox"/> UA Ukraine |
| <input checked="" type="checkbox"/> DK Denmark | <input checked="" type="checkbox"/> MA Morocco | <input checked="" type="checkbox"/> UG Uganda |
| <input checked="" type="checkbox"/> DM Dominica | <input checked="" type="checkbox"/> MD Republic of Moldova | <input checked="" type="checkbox"/> US United States of America |
| <input checked="" type="checkbox"/> DZ Algeria | | |
| <input checked="" type="checkbox"/> EC Ecuador | <input checked="" type="checkbox"/> MG Madagascar | <input checked="" type="checkbox"/> UZ Uzbekistan |
| <input checked="" type="checkbox"/> EE Estonia | <input checked="" type="checkbox"/> MK The former Yugoslav Republic of Macedonia | <input checked="" type="checkbox"/> VN Viet Nam |
| <input checked="" type="checkbox"/> ES Spain | <input checked="" type="checkbox"/> MN Mongolia | <input checked="" type="checkbox"/> YU Yugoslavia |
| <input checked="" type="checkbox"/> FI Finland | <input checked="" type="checkbox"/> MW Malawi | <input checked="" type="checkbox"/> ZA South Africa |
| <input checked="" type="checkbox"/> GB United Kingdom | | <input checked="" type="checkbox"/> ZW Zimbabwe |
| <input checked="" type="checkbox"/> GD Grenada | | |
| <input checked="" type="checkbox"/> GE Georgia | | |

Check-boxes below reserved for designating States which have become party to the PCT after issuance of this sheet:

- | | | |
|--|--|--------------------------|
| <input checked="" type="checkbox"/> PH Philippines | <input checked="" type="checkbox"/> ZM Zambia | <input type="checkbox"/> |
| <input checked="" type="checkbox"/> OM Oman | <input checked="" type="checkbox"/> TN Tunisia | <input type="checkbox"/> |

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. (Confirmation (including fees) must reach the receiving Office within the 15-month time limit.)

Supplemental Box

If the Supplemental Box is not used, this sheet should not be included in the request.

1. *If, in any of the Boxes, except Boxes Nos. VIII(f) to (v) for which a special continuation box is provided, the space is insufficient to furnish all the information: in such case, write "Continuation of Box No." (indicate the number of the Box) and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient, in particular:*
 - (i) *if more than two persons are to be indicated as applicants and/or inventors and no "continuation sheet" is available: in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below;*
 - (ii) *if, in Box No. II or in any of the sub-boxes of Box No. III, the indication "the States indicated in the Supplemental Box" is checked: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;*
 - (iii) *if, in Box No. II or in any of the sub-boxes of Box No. III, the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;*
 - (iv) *if, in addition to the agent(s) indicated in Box No. IV, there are further agents: in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;*
 - (v) *if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication "patent of addition," or "certificate of addition," or if, in Box No. V, the name of the United States of America is accompanied by an indication "continuation" or "continuation-in-part": in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;*
 - (vi) *if, in Box No. VI, there are more than five earlier applications whose priority is claimed: in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI.*
2. *If, with regard to the precautionary designation statement contained in Box No. V, the applicant wishes to exclude any State(s) from the scope of that statement: in such case, write "Designation(s) excluded from precautionary designation statement" and indicate the name or two-letter code of each State so excluded.*

ADDITIONAL REPRESENTATIVES:

PILCH, Adam John Michael
 CRISP, David Norman
 ROBINSON, Nigel Alexander Julian
 HARRIS, Ian Richard
 HARDING, Charles Thomas
 TURNER, James Arthur
 MALLALIEU, Catherine Louise
 PRATT, Richard Wilson
 HORNER, David Richard
 MASCHIO, Antonio
 POTTER, Julian Mark
 HAINES, Miles John
 PRICE, Paul Anthony King
 DEVILE, Jonathan Mark
 TANNER, James Percival
 KHOO, Chong-Yee
 HOLLIDAY, Louise Caroline
 MATHER, Belinda Jane
 MILLS, Julia
 HECTOR, Annabel Mary
 ALCOCK, David
 DAVIES, Simon Robert
 DENHOLM, Anna
 GALLAGHER, Kirk James
 WILLIAMS, Aylsa
 GODDARD, Frances Anna
 MCGOWAN, Cathrine
 MAIN, Malcolm

RO/GB
Added

Box No. VI PRIORITY CLAIM				
The priority of the following earlier application(s) is hereby claimed:				
Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is:		
		national application: country	regional application:* regional Office	international application: receiving Office
item (1) 20.4.01 ▲ 20 April 2001	0109781.5 /	GB		
item (2)				
item (3)				
item (4)				
item (5)				

☐ Further priority claims are indicated in the Supplemental Box.

The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) (only if the earlier application was filed with the Office which for the purposes of this international application is the receiving Office) identified above as:

☐ all items ☒ item (1) ☐ item (2) ☐ item (3) ☐ item (4) ☐ item (5) ☐ other, see Supplemental Box

* Where the earlier application is an ARIPO application, indicate at least one country party to the Paris Convention for the Protection of Industrial Property or one Member of the World Trade Organization for which that earlier application was filed (Rule 4.10(b)(ii)):

Box No. VII INTERNATIONAL SEARCHING AUTHORITY		
Choice of International Searching Authority (ISA) (if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):		
ISA /		
Request to use results of earlier search; reference to that search (if an earlier search has been carried out by or requested from the International Searching Authority):		
Date (day/month/year)	Number	Country (or regional Office)

Box No. VIII DECLARATIONS		
The following declarations are contained in Boxes Nos. VIII (i) to (v) (mark the applicable check-boxes below and indicate in the right column the number of each type of declaration):		Number of declarations
<input type="checkbox"/> Box No. VIII (i)	Declaration as to the identity of the inventor	:
<input type="checkbox"/> Box No. VIII (ii)	Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent	:
<input type="checkbox"/> Box No. VIII (iii)	Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application	:
<input type="checkbox"/> Box No. VIII (iv)	Declaration of inventorship (only for the purposes of the designation of the United States of America)	:
<input type="checkbox"/> Box No. VIII (v)	Declaration as to non-prejudicial disclosures or exceptions to lack of novelty	:

Box No. IX CHECK LIST; LANGUAGE OF FILING

This international application contains:		This international application is accompanied by the following item(s) (mark the applicable check-boxes below and indicate in right column the number of each item):	Number of items
(a) the following number of sheets in paper form:		1. <input checked="" type="checkbox"/> fee calculation sheet	:
request (including declaration sheets)	: 6	2. <input type="checkbox"/> original separate power of attorney	:
description (excluding sequence listing part)	: 57	3. <input type="checkbox"/> original general power of attorney	:
claims	: 4	4. <input type="checkbox"/> copy of general power of attorney; reference number, if any:	:
abstract	: 1	5. <input type="checkbox"/> statement explaining lack of signature	:
drawings	: 14	6. <input type="checkbox"/> priority document(s) identified in Box No. VI as item(s):	:
Sub-total number of sheets	: 82	7. <input type="checkbox"/> translation of international application into (language):	:
sequence listing part of description (actual number of sheets if filed in paper form, whether or not also filed in computer readable form; see (b) below)	: 10	8. <input type="checkbox"/> separate indications concerning deposited microorganism or other biological material	:
Total number of sheets	: 92	9. <input type="checkbox"/> sequence listing in computer readable form (indicate also type and number of carriers (diskette, CD-ROM, CD-R or other))	:
(b) sequence listing part of description filed in computer readable form		(i) <input type="checkbox"/> copy submitted for the purposes of international search under Rule 13ter only (and not as part of the international application)	:
(i) <input type="checkbox"/> only (under Section 801(a)(i))		(ii) <input type="checkbox"/> (only where check-box (b)(i) or (b)(ii) is marked in left column) additional copies including, where applicable, the copy for the purposes of international search under Rule 13ter	:
(ii) <input type="checkbox"/> in addition to being filed in paper form (under Section 801(a)(ii))		(iii) <input type="checkbox"/> together with relevant statement as to the identity of the copy or copies with the sequence listing part mentioned in left column	:
Type and number of carriers (diskette, CD-ROM, CD-R or other) on which the sequence listing part is contained (additional copies to be indicated under item 9(ii), in right column):		10. <input type="checkbox"/> other (specify):	:
Figure of the drawings which should accompany the abstract:		Language of filing of the international application: English	

Box No. X SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Catherine Mallaher C. MALLAHER
(Agent for the Applicants)

For receiving Office use only

1. Date of actual receipt of the purported international application:	19 APRIL 2002 19.4.02	2. Drawings: <input checked="" type="checkbox"/> received: <input type="checkbox"/> not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:		
4. Date of timely receipt of the required corrections under PCT Article 11(2):		
5. International Searching Authority (if two or more are competent): ISA /	6. <input type="checkbox"/> Transmittal of search copy delayed until search fee is paid	

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

VECTOR SYSTEM

5 FIELD OF THE INVENTION

The present invention relates to a vector system. In particular, the present invention relates to viral vector system capable of delivering a nucleotide sequence of interest ("NOI") to a target adipose tissue site.

10

BACKGROUND TO THE INVENTION

Adipose tissue has a major endocrine role in addition to its triglyceride storage role.

15 The most important endocrine role of adipose tissue is probably the secretion of leptin, however other factors such as sex steroids and glucocorticoids, peptide hormone precursors (eg angiotensinogen), complement factors, pro-inflammatory cytokines, interleukin 6, transforming growth factor-beta, tissue factor, plasminogen activator inhibitor-1 Aipo Q and adiponectin are also secreted. Aberrant function of
20 this tissue is associated with diseases such as diabetes (related to low levels of leptin secretion).

The regulation or alteration of these secretory functions by gene delivery could be use to treat a varied range of diseases.

25

One problem with gene therapy approaches in the treatment of diseases associated with adipose tissue, is that differentiated cells, such as adipose tissue cells, are difficult to transfect and/or transduce.

30 Hence, it is desirable to find a mechanism for transient or stable transfer of regulated genes into adipose tissue cells.

Finally, it is desirable to provide a better therapeutic approach for the treatment and/or prevention of diseases associated with adipose tissue.

SUMMARY OF ASPECTS OF THE PRESENT INVENTION

5

The first aspect of the present invention relates to a novel use of a viral vector system for transducing/infecting a target adipose tissue site.

10 In another aspect of the present invention there is provided the use of a viral vector system to transduce a target adipose tissue site, wherein the viral vector is pseudotyped with a nucleotide sequence that encodes at least a part of an env protein.

15 According to another aspect of the present invention there is provided the use of a viral vector system to transduce a target adipose tissue site, wherein the viral vector is derivable from the group selected from a retrovirus, including a lentivirus, an adenovirus, an adeno-associated virus, a pox virus, a herpes virus and a baculovirus.

20 In another aspect of the present invention there is provided the use of a viral vector system to transduce/infect a target adipose tissue site, wherein the viral vector is derivable from the group selected from an adenovirus and an adeno-associated virus.

25 According to another aspect of the present invention there is provided a method of treating and/or preventing a disease in a subject in need of same, said method comprising the step of using a viral vector system according to the present invention to transduce/infect a target adipose tissue site.

According to another aspect of the present invention there is provided a method for analysing the effect of a protein of interest ("POI") in a target adipose tissue site comprising the step of using a viral vector system according to the present invention.

30

In another aspect of the present invention there is provided a method for analysing the function of a gene, or a protein encoded by a gene, in a target cell, which method

comprises the step of inhibiting or blocking the expression of the gene using a viral vector system of the present invention.

According to another aspect of the present invention there is provided a target adipose tissue cell transduced with a viral vector system of the present invention.

In another aspect of the present invention there is provided the use of a target adipose tissue cell transduced/infected with a viral vector system of the present invention in the manufacture of a medicament for use in the prevention and/or treatment of a condition associated with adipose tissue metabolism.

According to another aspect of the present invention there is provided a method for treating and/or preventing a disease in a subject in need of same, said method comprising the step of transplanting a target adipose tissue cell transduced/infected with a viral vector system of the present invention into said subject.

In this respect, it has been found that a particular type of viral vector system is capable of effectively transducing a target adipose tissue site.

Other aspects of the present invention are presented in the accompanying claims and in the following description and drawings. These aspects are presented under separate section headings. However, it is to be understood that the teachings under each section are not necessarily limited to that particular section heading.

ADVANTAGES

The use of a viral vector system to transduce an adipose tissue site is advantageous because:

- (i) the adipose cells are long lived so that treatments may only be required infrequently;
- (ii) adipose tissue cells have a good connection to the circulatory system, so products could be quickly and efficiently supplied to the target organs;

- (iii) native adipose tissue cells can express the correct transport and processing functions so that it is more likely that functional proteins will be produced;
- (iv) if a gene mutation is involved in the disease phenotype, then expression in the native tissue site may allow product regulation by native systems; and
- 5 (v) gross regulation of product production may to some extent be determined by the numbers of cells treated.

Other advantages are discussed and are made apparent in the following commentary.

10 DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a new use of a viral vector system for transducing a target adipose tissue site.

- 15 Preferably the viral vectors of the present invention are recombinant viral vectors. Suitable recombinant viral vectors include but are not limited to retrovirus vectors, adenovirus vectors, adeno-associated viral (AAV) vectors, herpes-virus vectors, lentiviral vectors, baculoviral vectors, pox viral vectors or parvovirus vectors (see Kestler *et al* 1999 Human Gene Ther 10(10):1619-32).

20

In one embodiment of the present invention, a lentiviral vector is used to transduce a target adipose tissue site. A lentiviral vector belongs to the family of viral vectors known as retroviruses.

25 RETROVIRUSES

The concept of using retroviral vectors for gene therapy and gene delivery is well known (Verma and Somia (1997) Nature 389:239-242).

- 30 There are many retroviruses. For the present application, the term "retrovirus" includes: murine leukemia virus (MLV), human immunodeficiency virus (HIV), equine infectious anaemia virus (EIAV), mouse mammary tumour virus (MMTV), Rous sarcoma virus (RSV), Fujinami sarcoma virus (FuSV), Moloney murine

leukemia virus (Mo-MLV), FBR murine osteosarcoma virus (FBR MSV), Moloney murine sarcoma virus (Mo-MSV), Abelson murine leukemia virus (A-MLV), Avian myelocytomatosis virus-29 (MC29), and Avian erythroblastosis virus (AEV) and all other retroviridae including lentiviruses.

5

A detailed list of retroviruses may be found in Coffin *et al* ("Retroviruses" 1997 Cold Spring Harbour Laboratory Press Eds: JM Coffin, SM Hughes, HE Varmus pp 758-763).

- 10 In a preferred embodiment, the retroviral vector system is derivable from a lentivirus. Lentiviruses also belong to the retrovirus family, but they can infect both dividing and non-dividing cells (Lewis et al (1992) EMBO J. 3053-3058).

The lentivirus group can be split into "primate" and "non-primate". Examples of
 15 primate lentiviruses include the human immunodeficiency virus (HIV), the causative agent of human acquired immunodeficiency syndrome (AIDS), and the simian immunodeficiency virus (SIV). The non-primate lentiviral group includes the prototype "slow virus" visna/maedi virus (VMV), as well as the related caprine arthritis-encephalitis virus (CAEV), equine infectious anaemia virus (EIAV) and the
 20 more recently described feline immunodeficiency virus (FIV) and bovine immunodeficiency virus (BIV).

Details on the genomic structure of some lentiviruses may be found in the art. By way of example, details on HIV and EIAV may be found from the NCBI Genbank
 25 database (i.e. Genome Accession Nos. AF033819 and AF033820 respectively). Details of HIV variants may also be found at <http://hiv-web.lanl.gov>. Details of EIAV variants may be found through <http://www.ncbi.nlm.nih.gov>.

During the process of infection, a retrovirus initially attaches to a specific cell surface
 30 receptor. On entry into the susceptible host cell, the retroviral RNA genome is then copied to DNA by the virally encoded reverse transcriptase which is carried inside the parent virus. This DNA is transported to the host cell nucleus where it subsequently integrates into the host genome. At this stage, it is typically referred to as the

provirus. The provirus is stable in the host chromosome during cell division and is transcribed like other cellular genes. The provirus encodes the proteins and other factors required to make more virus, which can leave the cell by a process sometimes called “budding”.

5

Each retroviral genome comprises genes called *gag*, *pol* and *env* which code for virion proteins and enzymes. These genes are flanked at both ends by regions called long terminal repeats (LTRs). The LTRs are responsible for proviral integration, and transcription. They also serve as enhancer-promoter sequences. In other words, the

10 LTRs can control the expression of the viral genes. Encapsidation of the retroviral RNAs occurs by virtue of a *psi* sequence located at the 5' end of the viral genome.

The LTRs themselves are identical sequences that can be divided into three elements, which are called U3, R and U5. U3 is derived from the sequence unique to the 3' end

15 of the RNA. R is derived from a sequence repeated at both ends of the RNA and U5 is derived from the sequence unique to the 5' end of the RNA. The sizes of the three elements can vary considerably among different retroviruses.

For the viral genome, the site of transcription initiation is at the boundary between U3

20 and R in one LTR and the site of poly (A) addition (termination) is at the boundary between R and U5 in the other LTR. U3 contains most of the transcriptional control elements of the provirus, which include the promoter and multiple enhancer sequences responsive to cellular and in some cases, viral transcriptional activator proteins. Some retroviruses have any one or more of the following genes that code

25 for proteins that are involved in the regulation of gene expression: *tat*, *rev*, *tax* and *rex*.

With regard to the structural genes *gag*, *pol* and *env* themselves, *gag* encodes the internal structural protein of the virus. Gag protein is proteolytically processed into

30 the mature proteins MA (matrix), CA (capsid) and NC (nucleocapsid). The *pol* gene encodes the reverse transcriptase (RT), which contains DNA polymerase, associated RNase H and integrase (IN), which mediate replication of the genome. The *env* gene encodes the surface (SU) glycoprotein and the transmembrane (TM) protein of the

virion, which form a complex that interacts specifically with cellular receptor proteins. This interaction leads ultimately to infection by fusion of the viral membrane with the cell membrane.

- 5 Retroviruses may also contain “additional” genes which code for proteins other than gag, pol and env. Examples of additional genes include in HIV, one or more of *vif*, *vpr*, *vpx*, *vpu*, *tat*, *rev* and *nef*. EIAV has (amongst others) the additional gene S2.

- 10 Proteins encoded by additional genes serve various functions, some of which may be duplicative of a function provided by a cellular protein. In EIAV, for example, *tat* acts as a transcriptional activator of the viral LTR. It binds to a stable, stem-loop RNA secondary structure referred to as TAR. Rev regulates and co-ordinates the expression of viral genes through rev-response elements (RRE). The mechanisms of action of these two proteins are thought to be broadly similar to the analogous mechanisms in
15 the primate viruses. The function of S2 is unknown. In addition, an EIAV protein, Ttm, has been identified that is encoded by the first exon of *tat* spliced to the *env* coding sequence at the start of the transmembrane protein.

ADENOVIRUS VECTORS

20

In another embodiment of the present invention, an adenoviral vector system is used to transduce a target adipose tissue site.

- 25 By way of background information, the adenovirus is a double-stranded, linear DNA virus that does not go through an RNA intermediate. There are over 50 different human serotypes of adenovirus divided into 6 subgroups based on the genetic sequence homology. The natural target of adenovirus is the respiratory and gastrointestinal epithelia, generally giving rise to only mild symptoms. Serotypes 2 and 5 (with 95% sequence homology) are most commonly used in adenoviral vector
30 systems and are normally associated with upper respiratory tract infections in the young.

Adenoviruses are nonenveloped, regular icosohedrons. A typical adenovirus comprises a 140nm encapsidated DNA virus. The icosahedral symmetry of the virus is composed of 152 capsomeres: 240 hexons and 12 pentons. The core of the particle contains the 36kb linear duplex DNA which is covalently associated at the 5' ends with the Terminal Protein (TP) which acts as a primer for DNA replication. The DNA has inverted terminal repeats (ITR) and the length of these varies with the serotype.

Entry of adenovirus into cells involves a series of distinct events. Attachment of the virus to the cell occurs via an interaction between the viral fibre (37nm) and the fibre receptors on the cell. This receptor has recently been identified for Ad2/5 serotypes and designated as CAR (Coxsackie and Adeno Receptor, Tomko *et al* (1997 Proc Natl Acad Sci 94: 3352-2258). Internalisation of the virus into the endosome via the cellular $\alpha\beta 3$ and $\alpha\beta 5$ integrins is mediated by and viral RGD sequence in the penton-base capsid protein (Wickham *et al.*, 1993 Cell 73: 309-319). Following internalisation, the endosome is disrupted by a process known as endosomolysis, an event which is believed to be preferentially promoted by the cellular $\alpha\beta 5$ integrin (Wickham *et al.*, 1994 J Cell Biol 127: 257-264). In addition, there is recent evidence that the Ad5 fibre knob binds with high affinity to the MHC class 1 $\alpha 2$ domain at the surface of certain cell types including human epithelial and B lymphoblast cells (Hong *et al.*, 1997 EMBO 16: 2294-2306).

Subsequently the virus is translocated to the nucleus where activation of the early regions occurs and is shortly followed by DNA replication and activation of the late regions. Transcription, replication and packaging of the adenoviral DNA requires both host and viral functional protein machinery.

Viral gene expression can be divided into early (E) and late (L) phases. The late phase is defined by the onset of viral DNA replication. Adenovirus structural proteins are generally synthesised during the late phase. Following adenovirus infection, host cellular mRNA and protein synthesis is inhibited in cells infected with most serotypes. The adenovirus lytic cycle with adenovirus 2 and adenovirus 5 is very efficient and results in approximately 10, 000 virions per infected cell along with the synthesis of

excess viral protein and DNA that is not incorporated into the virion. Early adenovirus transcription is a complicated sequence of interrelated biochemical events but it entails essentially the synthesis of viral RNAs prior to the onset of DNA replication.

5

The Schematic diagram presented in Figure 11 is of the adenovirus genome showing the relative direction and position of early and late gene transcription:

The organisation of the adenovirus genome is similar in all of the adenovirus groups and specific functions are generally positioned at identical locations for each serotype studied. Early cytoplasmic messenger RNAs are complementary to four defined, noncontiguous regions on the viral DNA. These regions are designated E1-E4. The early transcripts have been classified into an array of intermediate early (E1a), delayed early (E1b, E2a, E2b, E3 and E4), and intermediate regions.

15 The early genes are expressed about 6-8 hours after infection and are driven from 7 promoters in gene blocks E1-4.

The E1a region is involved in transcriptional transactivation of viral and cellular genes as well as transcriptional repression of other sequences. The E1a gene exerts an important control function on all of the other early adenovirus messenger RNAs. In normal tissues, in order to transcribe regions E1b, E2a, E2b, E3 or E4 efficiently, active E1a product is required. However, the E1a function may be bypassed. Cells may be manipulated to provide E1a-like functions or may naturally contain such functions. The virus may also be manipulated to bypass the E1a function. The viral packaging signal overlaps with the E1a enhancer (194-358 nt).

25

The E1b region influences viral and cellular metabolism and host protein shut-off. It also includes the gene encoding the pIX protein (3525-4088 nt) which is required for packaging of the full length viral DNA and is important for the thermostability of the virus. The E1b region is required for the normal progression of viral events late in

30

infection. The E1b product acts in the host nucleus. Mutants generated within the E1b sequences exhibit diminished late viral mRNA accumulation as well as impairment in the inhibition of host cellular transport normally observed late in adenovirus infection. E1b is required for altering functions of the host cell such that processing and transport are shifted in favour of viral late gene products. These products then result in viral packaging and release of virions. E1b produces a 19 kD protein that prevents apoptosis. E1b also produces a 55 kD protein that binds to p53. For a review on adenoviruses and their replication, see WO 96/17053.

The E2 region is essential as it encodes the 72 kDa DNA binding protein, DNA polymerase and the 80 kDa precursor of the 55 kDa Terminal Protein (TP) needed for protein priming to initiate DNA synthesis.

A 19 kDa protein (gp19K) is encoded within the E3 region and has been implicated in modulating the host immune response to the virus. Expression of this protein is upregulated in response to TNF alpha during the first phase of the infection and this then binds and prevents migration of the MHC class I antigens to the epithelial surface, thereby dampening the recognition of the adenoviral infected cells by the cytotoxic T lymphocytes. The E3 region is dispensable in *in vitro* studies and can be removed by deletion of a 1.9 kb *Xba*I fragment.

The E4 region is concerned with decreasing the host protein synthesis and increasing the DNA replication of the virus.

There are 5 families of late genes and all are initiated from the major late promoter. The expression of the late genes includes a very complex post-transcriptional control mechanism involving RNA splicing. The fibre protein is encoded within the L5 region. The adenoviral genome is flanked by the inverted terminal repeat which in Ad5 is 103 bp and is essential for DNA replication. 30-40 hours post infection viral production is complete.

Adenoviruses may be converted for use as vectors for gene transfer by deleting the E1 gene, which is important for the induction of the E2, E3 and E4 promoters. The E1-replication defective virus may be propagated in a cell line that provides the E1

polypeptides in trans, such as the human embryonic kidney cell line 293. A therapeutic gene or genes can be inserted by recombination in place of the E1 gene. Expression of the gene is driven from either the E1 promoter or a heterologous promoter.

5

Even more attenuated adenoviral vectors have been developed by deleting some or all of the E4 open reading frames (ORFs). However, certain second generation vectors appear not to give longer-term gene expression, even though the DNA seems to be maintained. Thus, it appears that the function of one or more of the E4 ORFs may be
10 to enhance gene expression from at least certain viral promoters carried by the virus.

An alternative approach to making a more defective virus has been to “gut” the virus completely maintaining only the terminal repeats required for viral replication. The “guttled” or “gutless” viruses can be grown to high titres with a first generation helper
15 virus in the 293 cell line but it has been difficult to separate the “guttled” vector from the helper virus.

The adenovirus provides advantages as a vector for gene delivery.

20 It is a double stranded DNA nonenveloped virus that is capable of *in vivo* and *in vitro* transduction of a broad range of cell types of human and non-human origin. These cells include respiratory airway epithelial cells, hepatocytes, muscle cells, cardiac myocytes, synoviocytes, primary mammary epithelial cells and post-mitotically terminally differentiated cells such as neurons.

25

Adenoviral vectors are also capable of transducing non dividing cells. This is very important for diseases, such as cystic fibrosis, in which the affected cells in the lung epithelium, have a slow turnover rate. In fact, several trials are underway utilising adenovirus-mediated transfer of cystic fibrosis transporter (CFTR) into the lungs of
30 afflicted adult cystic fibrosis patients.

Adenoviruses have been used as vectors for gene therapy and for expression of heterologous genes. The large (36 kilobase) genome can accommodate up to 8kb of

foreign insert DNA and is able to replicate efficiently in complementing cell lines to produce very high titres of up to 10^{12} . Adenovirus is thus one of the best systems to study the expression of genes in primary non-replicative cells.

- 5 The expression of viral or foreign genes from the adenovirus genome does not require a replicating cell. Adenoviral vectors enter cells by receptor mediated endocytosis. Once inside the cell, adenovirus vectors rarely integrate into the host chromosome. Instead, it functions episomally (independently from the host genome) as a linear genome in the host nucleus. Hence the use of recombinant adenovirus alleviates the
10 problems associated with random integration into the host genome.

- In one embodiment of the present invention, the features of adenoviruses may be combined with the genetic stability of lentiviruses which can be used to transduce target adipose tissue cells to become transient lentiviral producer cells capable of
15 stably infect neighbouring cells. Such lentiviral producer cells which are engineered to express an NOI of the present invention can be implanted in organisms such as animals or humans for use in the treatment of disease such as cancer. Adenoviral delivery systems have been described in WO 00/17371.

20 POX VIRUSES

In a further embodiment of the present invention, a poxviral vector system is used to transduce an adipose tissue site.

- 25 Pox viral vectors may be used in accordance with the present invention, as large fragments of DNA are easily cloned into its genome and recombinant attenuated vaccinia variants have been described (Meyer, *et al.*, 1991, J. Gen. Virol. 72: 1031-1038, Smith and Moss 1983 Gene, 25:21-28). Pox viral vector delivery systems have been described in WO 00/29428.

30

Examples of pox viral vectors include but are not limited to leporipoxvirus: Upton, *et al* J. Virology 60:920 (1986) (Shope fibroma virus); capripoxvirus: Gershon, *et al* J. Gen. Virol. 70:525 (1989) (Kenya sheep-1); orthopoxvirus: Weir, *et al* J. Virol 46:530

(1983) (vaccinia); Esposito, *et al* Virology 135:561 (1984) (monkeypox and variola virus); Hruby, *et al* PNAS, 80:3411 (1983) (vaccinia); Kilpatrick, *et al* Virology 143:399 (1985) (Yaba monkey tumour virus); avipoxvirus: Binns, *et al* J. Gen. Virol 69:1275 (1988) (fowlpox); Boyle, *et al* Virology 156:355 (1987) (fowlpox); Schnitzlein, *et al* J. Virological Method, 20:341 (1988) (fowlpox, quailpox); entomopox (Lytvyn, *et al* J. Gen. Virol 73:3235-3240 (1992)).

Poxvirus vectors are used extensively as expression vehicles for genes of interest in eukaryotic cells. Their ease of cloning and propagation in a variety of host cells has led, in particular, to the widespread use of poxvirus vectors for expression of foreign protein and as delivery vehicles for vaccine antigens (Moss, B. 1991, Science 252: 1662-7).

Preferred vectors for use in accordance with the present invention are recombinant pox viral vectors such as fowl pox virus (FPV), entomopox virus, vaccinia virus such as NYVAC, canarypox virus, MVA or other non-replicating viral vector systems such as those described for example in WO 95/30018. Pox virus vectors have also been described where at least one immune evasion gene has been deleted (see WO 00/29428).

20

In one preferred embodiment, the pox virus vector is an entomopox virus vector.

VACCINIA VIRAL VECTORS

25 Preferably the pox viral vector is a vaccinia viral vector.

Preferably, the vector is a vaccinia virus vector such as MVA or NYVAC. Most preferred is the vaccinia strain modified virus ankara (MVA) or a strain derived therefrom. Alternatives to vaccinia vectors include avipox vectors such as fowlpox or canarypox known as ALVAC and strains derived therefrom which can infect and express recombinant proteins in human cells but are unable to replicate.

30

Preferred vectors for use in accordance with the present invention are recombinant pox viral vectors such as fowl pox virus (FPV), entomopox virus, vaccinia virus such as NYVAC, canarypox virus, MVA or other non-replicating viral vector systems such as those described for example in WO 95/30018.

5 VECTOR SYSTEMS

Retroviral vector systems, such as lentiviral vector systems have been proposed as a delivery system for *inter alia* the transfer of a NOI to one or more sites of interest. The transfer can occur *in vitro*, *ex vivo*, *in vivo*, or combinations thereof. Retroviral vector
10 systems have even been exploited to study various aspects of the retrovirus life cycle, including receptor usage, reverse transcription and RNA packaging (reviewed by Miller, 1992 Curr Top Microbiol Immunol 158:1-24).

As used herein the term “vector system” means a vector particle capable of
15 transducing a recipient cell with an NOI.

A vector particle includes the following components: a vector genome, which may contain one or more NOIs, a nucleocapsid encapsidating the nucleic acid, and a membrane surrounding the nucleocapsid.
20

The term “nucleocapsid” refers to at least the group specific viral core proteins (gag) and the viral polymerase (pol) of a retrovirus genome. These proteins encapsidate the packagable sequences and are themselves further surrounded by a membrane containing an envelope glycoprotein.
25

Once within the cell, the RNA genome from a retroviral vector particle is reverse transcribed into DNA and integrated into the DNA of the recipient cell.

The term “vector genome” refers to both to the RNA construct present in the retroviral vector particle and the integrated DNA construct. The term also embraces a
30 separate or isolated DNA construct capable of encoding such an RNA genome. A retroviral or lentiviral genome should comprise at least one component part derivable from a retrovirus or a lentivirus. The term “derivable” is used in its normal sense as

meaning a nucleotide sequence or a part thereof which need not necessarily be obtained from a retrovirus such as a lentivirus but instead could be derived therefrom. By way of example, the sequence may be prepared synthetically or by use of recombinant DNA techniques. Preferably the genome comprises a *psi* region (or an analogous component which is capable of causing encapsidation).

The viral vector genome is preferably “replication defective” by which we mean that the genome does not comprise sufficient genetic information alone to enable independent replication to produce infectious viral particles within the recipient cell. In a preferred embodiment, the genome lacks a functional *env*, *gag* or *pol* gene. If a highly preferred embodiment the genome lacks *env*, *gag* and *pol* genes.

The viral vector genome may comprise some or all of the long terminal repeats (LTRs). Preferably the genome comprises at least part of the LTRs or an analogous sequence which is capable of mediating proviral integration, and transcription. The sequence may also comprise or act as an enhancer-promoter sequence.

It is known that the separate expression of the components required to produce a retroviral vector particle on separate DNA sequences cointroduced into the same cell will yield retroviral particles carrying defective retroviral genomes that carry therapeutic genes (e.g. Reviewed by Miller 1992). This cell is referred to as the producer cell (see below).

There are two common procedures for generating producer cells. In one, the sequences encoding retroviral Gag, Pol and Env proteins are introduced into the cell and stably integrated into the cell genome; a stable cell line is produced which is referred to as the packaging cell line. The packaging cell line produces the proteins required for packaging retroviral RNA but it cannot bring about encapsidation due to the lack of a *psi* region. However, when a vector genome (having a *psi* region) is introduced into the packaging cell line, the helper proteins can package the *psi*-positive recombinant vector RNA to produce the recombinant virus stock. This can be used to transduce the NOI into recipient cells. The recombinant virus whose genome lacks all genes required to make viral proteins can infect only once and cannot propagate. Hence, the

NOI is introduced into the host cell genome without the generation of potentially harmful retrovirus. A summary of the available packaging lines is presented in "Retroviruses" (1997 Cold Spring Harbour Laboratory Press Eds: JM Coffin, SM Hughes, HE Varmus pp 449).

5

The present invention also provides a packaging cell line comprising a viral vector genome which is capable of producing a vector system useful in the first aspect of the invention. For example, the packaging cell line may be transduced with a viral vector system comprising the genome or transfected with a plasmid carrying a DNA construct
10 capable of encoding the RNA genome. The present invention also provides a kit for producing a viral vector system useful in the first aspect of the invention which comprises a packaging cell and a viral vector genome.

The second approach is to introduce the three different DNA sequences that are required
15 to produce a viral vector particle, such as a lentiviral vector particle i.e. the *env* coding sequences, the *gag-pol* coding sequence and the defective lentiviral genome containing one or more NOIs into the cell at the same time by transient transfection and the procedure is referred to as transient triple transfection (Landau & Littman 1992; Pear et al 1993). The triple transfection procedure has been optimised (Soneoka et al 1995;
20 Finer et al 1994). WO 94/29438 describes the production of producer cells *in vitro* using this multiple DNA transient transfection method. WO 97/27310 describes a set of DNA sequences for creating lentiviral producer cells either *in vivo* or *in vitro* for re-implantation.

25 Transient transfection has numerous advantages over the packaging cell method. In this regard, transient transfection avoids the longer time required to generate stable vector-producing cell lines and is used if the vector genome or retroviral packaging components are toxic to cells. If the vector genome encodes toxic genes or genes that interfere with the replication of the host cell, such as inhibitors of the cell cycle or
30 genes that induce apoptosis, it may be difficult to generate stable vector-producing cell lines, but transient transfection can be used to produce the vector before the cells die. Also, cell lines have been developed using transient infection that produce vector

titre levels that are comparable to the levels obtained from stable vector-producing cell lines (Pear et al 1993, PNAS 90:8392-8396).

5 The components of the viral system which are required to complement the vector genome may be present on one or more “producer plasmids” for transfecting into cells.

The present invention also provides a kit for producing a viral vector system useful in the first aspect of the invention, comprising

- 10 (i) a viral vector genome which is incapable of encoding one or more proteins which are required to produce a vector particle;
- (ii) one or more producer plasmid(s) capable of encoding the protein which is not encoded by (i); and optionally
- (iii) a cell suitable for conversion into a producer cell.

15 In a preferred embodiment, the viral vector genome is incapable of encoding the proteins gag, pol and env. Preferably the kit comprises one or more producer plasmids encoding env, gag and pol, for example, one producer plasmid encoding env and one encoding gag-pol. Preferably the gag-pol sequence is codon optimised for use in the particular producer cell (see below).

20

The present invention also provides a producer cell expressing the vector genome and the producer plasmid(s) capable of producing a viral vector system useful in the present invention.

25 Preferably the viral vector system used in the first aspect of the present invention is a self-inactivating (SIN) vector system.

By way of example, self-inactivating retroviral vector systems have been constructed by deleting the transcriptional enhancers or the enhancers and promoter in the U3
30 region of the 3' LTR. After a round of vector reverse transcription and integration, these changes are copied into both the 5' and the 3' LTRs producing a transcriptionally inactive provirus. However, any promoter(s) internal to the LTRs in such vectors will still be transcriptionally active. This strategy has been employed to

eliminate effects of the enhancers and promoters in the viral LTRs on transcription from internally placed genes. Such effects include increased transcription or suppression of transcription. This strategy can also be used to eliminate downstream transcription from the 3' LTR into genomic DNA. This is of particular concern in human gene therapy where it may be important to prevent the adventitious activation of an endogenous oncogene.

Preferably a recombinase assisted mechanism is used which facilitates the production of high titre regulated lentiviral vectors from the producer cells of the present invention.

As used herein, the term "recombinase assisted system" includes but is not limited to a system using the Cre recombinase / loxP recognition sites of bacteriophage P1 or the site-specific FLP recombinase of *S. cerevisiae* which catalyses recombination events between 34 bp FLP recognition targets (FRTs).

The site-specific FLP recombinase of *S. cerevisiae* which catalyses recombination events between 34 bp FLP recognition targets (FRTs) has been configured into DNA constructs in order to generate high level producer cell lines using recombinase-assisted recombination events (Karreman et al (1996) NAR 24:1616-1624). A similar system has been developed using the Cre recombinase / loxP recognition sites of bacteriophage P1 (see PCT/GB00/03837; Vanin et al (1997) J. Virol 71:7820-7826). This was configured into a lentiviral genome such that high titre lentiviral producer cell lines were generated.

By using producer/packaging cell lines, it is possible to propagate and isolate quantities of viral vector particles (e.g. to prepare suitable titres of the viral vector particles) for subsequent transduction of, for example, a site of interest (such as adult brain tissue). Producer cell lines are usually better for large scale production or vector particles.

Producer cells/packaging cells can be of any suitable cell type. Producer cells are generally mammalian cells but can be, for example, insect cells.

As used herein, the term “producer cell” or “vector producing cell” refers to a cell which contains all the elements necessary for production of retroviral vector particles. Preferably, the producer cell is obtainable from a stable producer cell line. Preferably, the producer cell is obtainable from a derived stable producer cell line.

5

Preferably, the producer cell is obtainable from a derived producer cell line.

As used herein, the term “derived producer cell line” is a transduced producer cell line which has been screened and selected for high expression of a marker gene. Such cell lines support high level expression from the viral genome. The term “derived producer cell line” is used interchangeably with the term “derived stable producer cell line” and the term “stable producer cell line.”

10

Preferably the derived producer cell line includes but is not limited to a retroviral and/or a lentiviral producer cell.

15

Preferably the derived producer cell line is an HIV or EIAV producer cell line, more preferably an EIAV producer cell line.

Preferably the envelope protein sequences, and nucleocapsid sequences are all stably integrated in the producer and/or packaging cell. However, one or more of these sequences could also exist in episomal form and gene expression could occur from the episome.

20

As used herein, the term “packaging cell” refers to a cell which contains those elements necessary for production of infectious recombinant virus which are lacking in the RNA genome. Typically, such packaging cells contain one or more producer plasmids which are capable of expressing viral structural proteins (such as *gag-pol* and *env*, which may be codon optimised) but they do not contain a packaging signal.

25

30

The term “packaging signal” which is referred to interchangeably as “packaging sequence” or “*psi*” is used in reference to the non-coding, *cis*-acting sequence required for encapsidation of retroviral RNA strands during viral particle formation.

In HIV-1, this sequence has been mapped to loci extending from upstream of the major splice donor site (SD) to at least the *gag* start codon.

5 Packaging cell lines may be readily prepared (see also WO 92/05266), and utilised to create producer cell lines for the production of retroviral vector particles. As already mentioned above, a summary of the available packaging lines is presented in "Retroviruses".

10 Also as discussed above, simple packaging cell lines, comprising a provirus in which the packaging signal has been deleted, have been found to lead to the rapid production of undesirable replication competent viruses through recombination. In order to improve safety, second generation cell lines have been produced wherein the 3'LTR of the provirus is deleted. In such cells, two recombinations would be necessary to produce a wild type virus. A further improvement involves the introduction of the
15 *gag-pol* genes and the *env* gene on separate constructs so-called third generation packaging cell lines. These constructs are introduced sequentially to prevent recombination during transfection.

Preferably, the packaging cell lines are second generation packaging cell lines.

20

Preferably, the packaging cell lines are third generation packaging cell lines.

25 In these split-construct, third generation cell lines, a further reduction in recombination may be achieved by changing the codons. This technique, based on the redundancy of the genetic code, aims to reduce homology between the separate constructs, for example between the regions of overlap in the *gag-pol* and *env* open reading frames.

30 The packaging cell lines are useful for providing the gene products necessary to encapsidate and provide a membrane protein for a high titre vector particle production. The packaging cell may be a cell cultured *in vitro* such as a tissue culture cell line. Suitable cell lines include but are not limited to mammalian cells such as

murine fibroblast derived cell lines or human cell lines. Preferably the packaging cell line is a human cell line, such as for example: HEK293, 293-T, TE671, HT1080.

Alternatively, the packaging cell may be a cell derived from the individual to be
5 treated such as a monocyte, macrophage, blood cell or fibroblast. The cell may be isolated from an individual and the packaging and vector components administered *ex vivo* followed by re-administration of the autologous packaging cells.

It is highly desirable to use high-titre virus preparations in both experimental and
10 practical applications. Techniques for increasing viral titre include using a *psi* plus packaging signal as discussed above and concentration of viral stocks.

As used herein, the term “high titre” means an effective amount of a viral vector or particle which is capable of transducing a target site such as a target adipose tissue
15 site.

As used herein, the term “effective amount” means an amount of a regulated viral or lentiviral vector or vector particle which is sufficient to induce expression of the NOIs at a target adipose tissue site.
20

A high-titre viral preparation for a producer/packaging cell is usually of the order of 10^5 to 10^7 t.u. per ml. (The titer is expressed in transducing units per ml (t.u./ml) as titred on a standard D17 cell line). For transduction in adipose tissues, it may be necessary to use very small volumes, so the viral preparation is concentrated by
25 ultracentrifugation, low-speed centrifugation or cross-slow filtration. The resulting preparation should have at least 10^8 t.u./ml, preferably from 10^8 to 10^9 t.u./ml, more preferably at least 10^9 t.u./ml.

The expression products encoded by the NOIs may be proteins which are secreted from the cell. Alternatively the NOI expression products are not secreted and are active within the cell. For some applications, it is preferred for the NOI expression product to demonstrate a bystander effect or a distant bystander effect; that is the production of the expression product in one cell leading to the modulation of

additional, related cells, either neighbouring or distant (e.g. metastatic), which possess a common phenotype.

The presence of a sequence termed the central polypurine tract (cPPT) may improve the efficiency of gene delivery to non-dividing cells (see WO 00/31200). This *cis*-acting element is located, for example, in the EIAV polymerase coding region
 5 element. Preferably the genome of the vector system used in the present invention comprises a cPPT sequence. In addition, or in the alternative, the viral genome may comprise a post-translational regulatory element and/or a translational enhancer.

The NOIs may be operatively linked to one or more promoter/enhancer elements.
 10 Transcription of one or more NOI may be under the control of viral LTRs or alternatively promoter-enhancer elements can be engineered in with the transgene. Preferably the promoter is a strong promoter such as CMV. The promoter may be a regulated promoter. The promoter may be tissue-specific.

15 MINIMAL SYSTEMS

It has been demonstrated that a primate lentivirus minimal system can be constructed which requires none of the HIV/SIV additional genes *vif*, *vpr*, *vpx*, *vpu*, *tat*, *rev* and *nef* for either vector production or for transduction of dividing and non-dividing cells.
 20 It has also been demonstrated that an EIAV minimal vector system can be constructed which does not require *S2* for either vector production or for transduction of dividing and non-dividing cells. The deletion of additional genes is highly advantageous. Firstly, it permits vectors to be produced without the genes associated with disease in lentiviral (e.g. HIV) infections. In particular, *tat* is associated with disease. Secondly,
 25 the deletion of additional genes permits the vector to package more heterologous DNA. Thirdly, genes whose function is unknown, such as *S2*, may be omitted, thus reducing the risk of causing undesired effects. Examples of minimal lentiviral vectors are disclosed in WO-A-99/32646 and in WO-A-98/17815.

30 Thus, preferably, the delivery system used in the invention is devoid of at least *tat* and *S2* (if it is an EIAV vector system), and possibly also *vif*, *vpr*, *vpx*, *vpu* and *nef*. More

preferably, the systems of the present invention are also devoid of *rev*. Rev was previously thought to be essential in some retroviral genomes for efficient virus production. For example, in the case of HIV, it was thought that *rev* and RRE sequence should be included. However, it has been found that the requirement for *rev* and RRE can be reduced or eliminated by codon optimisation (see below) or by replacement with other functional equivalent systems such as the MPMV system. As expression of the codon optimised *gag-pol* is REV independent, RRE can be removed from the *gag-pol* expression cassette, thus removing any potential for recombination with any RRE contained on the vector genome.

In a preferred embodiment the viral genome of the first aspect of the invention lacks the Rev response element (RRE).

In a preferred embodiment, the system used in the present invention is based on a so-called "minimal" system in which some or all of the additional genes have been removed.

CODON OPTIMISATION

Codon optimisation has previously been described in WO99/41397. Different cells differ in their usage of particular codons. This codon bias corresponds to a bias in the relative abundance of particular tRNAs in the cell type. By altering the codons in the sequence so that they are tailored to match with the relative abundance of corresponding tRNAs, it is possible to increase expression. By the same token, it is possible to decrease expression by deliberately choosing codons for which the corresponding tRNAs are known to be rare in the particular cell type. Thus, an additional degree of translational control is available.

Many viruses, including HIV and other lentiviruses, use a large number of rare codons and by changing these to correspond to commonly used mammalian codons, increased expression of the packaging components in mammalian producer cells can be achieved. Codon usage tables are known in the art for mammalian cells, as well as for a variety of other organisms.

Codon optimisation has a number of other advantages. By virtue of alterations in their sequences, the nucleotide sequences encoding the packaging components of the viral particles required for assembly of viral particles in the producer cells/packaging cells have RNA instability sequences (INS) eliminated from them. At the same time, the amino acid sequence coding sequence for the packaging components is retained so that the viral components encoded by the sequences remain the same, or at least sufficiently similar that the function of the packaging components is not compromised. Codon optimisation also overcomes the Rev/RRE requirement for export, rendering optimised sequences Rev independent. Codon optimisation also reduces homologous recombination between different constructs within the vector system (for example between the regions of overlap in the *gag-pol* and *env* open reading frames). The overall effect of codon optimisation is therefore a notable increase in viral titre and improved safety.

In one embodiment only codons relating to INS are codon optimised. However, in a much more preferred and practical embodiment, the sequences are codon optimised in their entirety, with the exception of the sequence encompassing the frameshift site.

The *gag-pol* gene comprises two overlapping reading frames encoding the *gag-pol* proteins. The expression of both proteins depends on a frameshift during translation. This frameshift occurs as a result of ribosome “slippage” during translation. This slippage is thought to be caused at least in part by ribosome-stalling RNA secondary structures. Such secondary structures exist downstream of the frameshift site in the *gag-pol* gene. For HIV, the region of overlap extends from nucleotide 1222 downstream of the beginning of *gag* (wherein nucleotide 1 is the A of the *gag* ATG) to the end of *gag* (nt 1503). Consequently, a 281 bp fragment spanning the frameshift site and the overlapping region of the two reading frames is preferably not codon optimised. Retaining this fragment will enable more efficient expression of the *gag-pol* proteins.

For EIAV the beginning of the overlap has been taken to be nt 1262 (where nucleotide 1 is the A of the *gag* ATG). The end of the overlap is at 1461 bp. In order to ensure

that the frameshift site and the *gag-pol* overlap are preserved, the wild type sequence has been retained from nt 1156 to 1465.

Derivations from optimal codon usage may be made, for example, in order to
5 accommodate convenient restriction sites, and conservative amino acid changes may be introduced into the *gag-pol* proteins.

In a highly preferred embodiment, codon optimisation was based on lightly expressed mammalian genes. The third and sometimes the second and third base may be
10 changed.

Due to the degenerate nature of the Genetic Code, it will be appreciated that numerous *gag-pol* sequences can be achieved by a skilled worker. Also there are many retroviral variants described which can be used as a starting point for generating a
15 codon optimised *gag-pol* sequence. Lentiviral genomes can be quite variable. For example there are many quasi-species of HIV-1 which are still functional. This is also the case for EIAV. These variants may be used to enhance particular parts of the transduction process. Examples of HIV-1 variants may be found at <http://hiv-web.lanl.gov>. Details of EIAV clones may be found at the NCBI database:
20 <http://www.ncbi.nlm.nih.gov>.

The strategy for codon optimised *gag-pol* sequences can be used in relation to any retrovirus. This would apply to all lentiviruses, including EIAV, FIV, BIV, CAEV, VMR, SIV, HIV-1 and HIV-2. In addition this method could be used to increase
25 expression of genes from HTLV-1, HTLV-2, HFV, HSRV and human endogenous retroviruses (HERV), MLV and other retroviruses.

Codon optimisation can render *gag-pol* expression Rev independent. In order to enable the use of anti-*rev* or RRE factors in the retroviral vector, however, it would be
30 necessary to render the viral vector generation system totally Rev/RRE independent. Thus, the genome also needs to be modified. This is achieved by optimising vector genome components. Advantageously, these modifications also lead to the production

of a safer system absent of all additional proteins both in the producer and in the transduced cell.

As described above, the packaging components for a retroviral vector include
 5 expression products of *gag*, *pol* and *env* genes. In addition, efficient packaging depends on a short sequence of 4 stem loops followed by a partial sequence from *gag* and *env* (the “packaging signal”). Thus, inclusion of a deleted *gag* sequence in the retroviral vector genome (in addition to the full *gag* sequence on the packaging construct) will optimise vector titre. To date efficient packaging has been reported to
 10 require from 255 to 360 nucleotides of *gag* in vectors that still retain *env* sequences, or about 40 nucleotides of *gag* in a particular combination of splice donor mutation, *gag* and *env* deletions. It has surprisingly been found that a deletion of all but the N-terminal 360 or so nucleotides in *gag* leads to an increase in vector titre. Thus, preferably, the retroviral vector genome includes a *gag* sequence which comprises one
 15 or more deletions, more preferably the *gag* sequence comprises about 360 nucleotides derivable from the N-terminus.

PSEUDOTYPING

20 In the design of viral vector systems, such as lentiviral vector systems, it is desirable to engineer particles with different target cell specificities to the native virus, to enable the delivery of genetic material to an expanded or altered range of cell types. One manner in which to achieve this is by engineering the virus envelope protein to alter its specificity. Another approach is to introduce a heterologous envelope protein
 25 into the vector particle to replace or add to the native envelope protein of the virus.

Thus, in accordance with a preferred embodiment, the viral vector system is pseudotyped.

30 The term pseudotyping means incorporating in at least a part of, or substituting a part of, or replacing all of, an *env* gene of a viral genome with a heterologous *env* gene, for example an *env* gene from another virus. Pseudotyping is not a new phenomenon and examples may be found in WO 99/61639, WO-A-98/05759, WO-A-98/05754, WO-

A-97/17457, WO-A-96/09400, WO-A-91/00047 and Mebatsion *et al* 1997 Cell 90, 841-847.

Pseudotyping can improve retroviral vector stability and transduction efficiency. A
 5 pseudotype of murine leukemia virus packaged with lymphocytic choriomeningitis
 virus (LCMV) has been described (Miletic *et al* (1999) J. Virol. 73:6114-6116) and
 shown to be stable during ultracentrifugation and capable of infecting several cell
 lines from different species. Other envelope proteins, such as envelope proteins from
 the Ebola virus may also be used.

10 In one embodiment of the present invention, the vector system may be pseudotyped
 with LMCV env protein.

In another embodiment of the present invention, the vector system may be
 15 pseudotyped with an envelope proteins from the Ebola virus.

In another embodiment of the present invention the viral vector system may be
 pseudotyped with at least a part of a rabies G protein or a mutant, variant, homologue
 or fragment thereof, or at least a part of a VSV G protein or a mutant, variant,
 20 homologue or fragment thereof, ^{or} at least a part of a ^{virus} ~~or~~ coccal glycoprotein (GenBank
 AF045556) or a mutant, variant, homologue or fragment thereof, or at least a part of a
 chandipura ^{virus} glycoprotein ^{GenBank} (J04350) or a mutant, variant, homologue or fragment
 thereof.

25 Thus, in one embodiment of the present invention, there is provided the use of a viral
 delivery system comprising a heterologous *env* region, wherein the heterologous *env*
 region comprises at least a part of a rabies G protein or a mutant, variant, homologue
 or fragment thereof, or at least a part of a VSV G protein or a mutant, variant,
 homologue or fragment thereof, at least a part of a or coccal glycoprotein or a mutant,
 30 variant, homologue or fragment thereof, or at least a part of a chandipura glycoprotein
 or a mutant, variant, homologue or fragment thereof to transduce a target adipose
 tissue site.

The heterologous env region may be encoded by a gene which is present on a producer plasmid. The producer plasmid may be present as part of a kit for the production of viral vector particles suitable for use in the first aspect of the invention.

5 RABIES G PROTEIN

In another embodiment of the present invention, the vector system may be pseudotyped with at least a part of a rabies G protein or a mutant, variant, homologue or fragment thereof.

10

Teachings on the rabies G protein, as well as mutants thereof, may be found in in WO 99/61639 and well as Rose *et al.*, 1982 J. Virol. 43: 361-364, Hanham *et al.*, 1993 J. Virol., 67, 530-542, Tuffereau *et al.*, 1998 J. Virol., 72, 1085-1091, Kucera *et al.*, 1985 J. Virol 55, 158-162, Dietzschold *et al.*, 1983 PNAS 80, 70-74, Seif *et al.*, 1985 J. Virol., 53, 926-934, Coulon *et al.*, 1998 J. Virol., 72, 273-278, Tuffereau *et al.*, 1998 J. Virol., 72, 1085-10910, Burger *et al.*, 1991 J. Gen. Virol. 72. 359-367, Gaudin *et al* 1995 J Virol 69, 5528-5534, Benmansour *et al* 1991 J Virol 65, 4198-4203, Luo *et al* 1998 Microbiol Immunol 42, 187-193, Coll 1997 Arch Virol 142, 2089-2097, Luo *et al* 1997 Virus Res 51, 35-41, Luo *et al* 1998 Microbiol Immunol 42, 187-193, Coll 1995 Arch Virol 140, 827-851, Tuchiya *et al* 1992 Virus Res 25, 1-13, Morimoto *et al* 1992 Virology 189, 203-216, Gaudin *et al* 1992 Virology 187, 627-632, Whitt *et al* 1991 Virology 185, 681-688, Dietzschold *et al* 1978 J Gen Virol 40, 131-139, Dietzschold *et al* 1978 Dev Biol Stand 40, 45-55, Dietzschold *et al* 1977 J Virol 23, 286-293, and Otvos *et al* 1994 Biochim Biophys Acta 1224, 68-76. A rabies G protein is also described in EP-A-0445625.

15
20
25

The use of rabies G protein provides vectors which, *in vivo*, preferentially transduce targeted cells which rabies virus preferentially infects. This includes adipose tissue target cells *in vivo*. For an adipose tissue targeted vector, rabies G from a pathogenic strain of rabies such as ERA may be particularly effective. On the other hand rabies G protein confers a wider target cell range *in vitro* including nearly all mammalian and avian cell types tested (Seganti *et al.*, 1990 Arch Virol. 34, 155-163; Fields *et al.*,

30

1996 Fields Virology, Third Edition, vol.2, Lippincott-Raven Publishers, Philadelphia, New York).

The tropism of the pseudotyped vector particles may be modified by the use of a mutant rabies G which is modified in the extracellular domain. Rabies G protein has the advantage of being mutable to restrict target cell range. The uptake of rabies virus by target cells *in vivo* is thought to be mediated by the acetylcholine receptor (AchR) but there may be other receptors to which it binds *in vivo* (Hanham *et al.*, 1993 J. Virol., 67, 530-542; Tuffereau *et al.*, 1998 J. Virol., 72, 1085-1091). It is thought that multiple receptors are used in the nervous system for viral entry, including NCAM (Thoulouze *et al.* (1998) J. Virol 72(9):7181-90) and p75 Neurotrophin receptor (Tuffereau *et al.* (1998) Embo J 17(24) 7250-9).

The effects of mutations in antigenic site III of the rabies G protein on virus tropism have been investigated, this region is not thought to be involved in the binding of the virus to the acetylcholine receptor (Kucera *et al.*, 1985 J. Virol 55, 158-162; Dietzschold *et al.*, 1983 Proc Natl Acad Sci 80, 70-74; Seif *et al.*, 1985 J. Virol., 53, 926-934; Coulon *et al.*, 1998 J. Virol., 72, 273-278; Tuffereau *et al.*, 1998 J. Virol., 72, 1085-1091). For example a mutation of the arginine at amino acid 333 in the mature protein to glutamine can be used to restrict viral entry to olfactory and peripheral neurons *in vivo* while reducing propagation to the central nervous system. These viruses were able to penetrate motor neurons and sensory neurons as efficiently as the wild type virus, yet transneuronal transfer did not occur (Coulon *et al.*, 1989, J. Virol. 63, 3550-3554). Viruses in which amino acid 330 has been mutated are further attenuated, being unable to infect either motor neurons or sensory neurons after intramuscular injection (Coulon *et al.*, 1998 J. Virol., 72, 273-278).

Alternatively or additionally, rabies G proteins from laboratory passaged strains of rabies may be used. These can be screened for alterations in tropism. Such strains include the following:

Genbank accession number	Rabies Strain
--------------------------	---------------

J02293	ERA
U52947	COSRV
U27214	NY 516
U27215	NY771
5 U27216	FLA125
U52946	SHBRV
M32751	HEP-Flury

- 10 By way of example, the ERA strain is a pathogenic strain of rabies and the rabies G protein from this strain can be used for transduction of neuronal cells. The sequence of rabies G from the ERA strains is in the GenBank database (accession number J02293). This protein has a signal peptide of 19 amino acids and the mature protein begins at the lysine residue 20 amino acids from the translation initiation methionine.
- 15 The HEP-Flury strain contains the mutation from arginine to glutamine at amino acid position 333 in the mature protein which correlates with reduced pathogenicity and which can be used to restrict the tropism of the viral envelope.

WO 99/61639 discloses the nucleic and amino acid sequences for a rabies virus strain
 20 ERA (Genbank locus RAVGPLS, accession M38452).

VSV-G PROTEIN

25 The envelope glycoprotein (G) of Vesicular stomatitis virus (VSV), a rhabdovirus, is another envelope protein that has been shown to be capable of pseudotyping certain retroviruses.

Its ability to pseudotype MoMLV- based retroviral vectors in the absence of any retroviral envelope proteins was first shown by Emi *et al* (1991 Journal of Virology 65:1202-1207). WO94/294440 teaches that retroviral vectors may be successfully
 30 pseudotyped with VSV-G. These pseudotyped VSV-G vectors may be used to transduce a wide range of mammalian cells. Even more recently, Abe et al (J Virol

1998 72(8) 6356-6361) teach that non-infectious retroviral particles can be made infectious by the addition of VSV-G.

Burns *et al* (1993 Proc. Natl. Acad. Sci. USA 90: 8033-7) successfully pseudotyped the retrovirus MLV with VSV-G and this resulted in a vector having an altered host range compared to MLV in its native form. VSV-G pseudotyped vectors have been shown to infect not only mammalian cells, but also cell lines derived from fish, reptiles and insects (Burns *et al* 1993 *ibid*). They have also been shown to be more efficient than traditional amphotropic envelopes for a variety of cell lines (Yee *et al*, 1994 Proc. Natl. Acad. Sci. USA 91: 9564-9568, Lin, Emi *et al*, 1991 Journal of Virology 65:1202-1207). VSV-G protein can be used to pseudotype certain retroviruses because its cytoplasmic tail is capable of interacting with the retroviral cores.

The provision of a non-retroviral pseudotyping envelope such as VSV-G protein gives the advantage that vector particles can be concentrated to a high titre without loss of infectivity (Akkina *et al*, 1996 J. Virol. 70: 2581-5). Retrovirus envelope proteins are apparently unable to withstand the shearing forces during ultracentrifugation, probably because they consist of two non-covalently linked subunits. The interaction between the subunits may be disrupted by the centrifugation. In comparison the VSV glycoprotein is composed of a single unit. VSV-G protein pseudotyping can therefore offer potential advantages.

WO 00/52188 describes the generation of pseudotyped retroviral vectors, from stable producer cell lines, having vesicular stomatitis virus-G protein (VSV-G) as the membrane-associated viral envelope protein, and provides a gene sequence for the VSV-G protein.

MUTANTS, VARIANTS, HOMOLOGUES AND FRAGMENTS

In one embodiment of the present invention, the viral vector system used in the present invention may be pseudotyped with a mutant, variant, homologue or fragment

of the wild-type Rabies G or VSV-G or chandipura glycoprotein or coccal glycoprotein protein.

5 The term “wild type” is used to mean an polypeptide having a primary amino acid sequence which is identical with the native protein (i.e., the viral protein).

10 The term “mutant” is used to mean a polypeptide having a primary amino acid sequence which differs from the wild type sequence by one or more amino acid additions, substitutions or deletions. A mutant may arise naturally, or may be created artificially (for example by site-directed mutagenesis). Preferably the mutant has at least 90% sequence identity with the wild type sequence. Preferably the mutant has 20 mutations or less over the whole wild-type sequence. More preferably the mutant has 10 mutations or less, most preferably 5 mutations or less over the whole wild-type sequence.

15

The term “variant” is used to mean a naturally occurring polypeptide which differs from a wild-type sequence. A variant may be found within the same viral strain (i.e. if there is more than one isoform of the protein) or may be found within a different strains. Preferably the variant has at least 90% sequence identity with the wild type sequence. Preferably the variant has 20 mutations or less over the whole wild-type sequence. More preferably the variant has 10 mutations or less, most preferably 5 mutations or less over the whole wild-type sequence.

25 Here, the term “homologue” means an entity having a certain homology with the wild type amino acid sequence and the wild type nucleotide sequence. Here, the term “homology” can be equated with “identity”.

30 In the present context, an homologous sequence is taken to include an amino acid sequence which may be at least 75, 85 or 90% identical, preferably at least 95 or 98% identical to the subject sequence. Typically, the homologues will comprise the same active sites etc. as the subject amino acid sequence. Although homology can also be considered in terms of similarity (i.e. amino acid residues having similar chemical

properties/functions), in the context of the present invention it is preferred to express homology in terms of sequence identity.

5 In the present context, an homologous sequence is taken to include a nucleotide sequence which may be at least 75, 85 or 90% identical, preferably at least 95 or 98% identical to the subject sequence. Typically, the homologues will comprise the same sequences that code for the active sites etc. as the subject sequence. Although homology can also be considered in terms of similarity (i.e. amino acid residues having similar chemical properties/functions), in the context of the present invention
10 it is preferred to express homology in terms of sequence identity.

Homology comparisons can be conducted by eye, or more usually, with the aid of readily available sequence comparison programs. These commercially available computer programs can calculate % homology between two or more sequences.

15

% homology may be calculated over contiguous sequences, i.e. one sequence is aligned with the other sequence and each amino acid in one sequence is directly compared with the corresponding amino acid in the other sequence, one residue at a time. This is called an “ungapped” alignment. Typically, such ungapped alignments
20 are performed only over a relatively short number of residues.

Although this is a very simple and consistent method, it fails to take into consideration that, for example, in an otherwise identical pair of sequences, one insertion or deletion will cause the following amino acid residues to be put out of alignment, thus
25 potentially resulting in a large reduction in % homology when a global alignment is performed. Consequently, most sequence comparison methods are designed to produce optimal alignments that take into consideration possible insertions and deletions without penalising unduly the overall homology score. This is achieved by inserting “gaps” in the sequence alignment to try to maximise local homology.

30

However, these more complex methods assign “gap penalties” to each gap that occurs in the alignment so that, for the same number of identical amino acids, a sequence alignment with as few gaps as possible - reflecting higher relatedness between the two

compared sequences - will achieve a higher score than one with many gaps. "Affine gap costs" are typically used that charge a relatively high cost for the existence of a gap and a smaller penalty for each subsequent residue in the gap. This is the most commonly used gap scoring system. High gap penalties will of course produce

5 optimised alignments with fewer gaps. Most alignment programs allow the gap penalties to be modified. However, it is preferred to use the default values when using such software for sequence comparisons. For example when using the GCG Wisconsin Bestfit package the default gap penalty for amino acid sequences is -12 for a gap and -4 for each extension.

10

Calculation of maximum % homology therefore firstly requires the production of an optimal alignment, taking into consideration gap penalties. A suitable computer program for carrying out such an alignment is the GCG Wisconsin Bestfit package (University of Wisconsin, U.S.A.; Devereux *et al.*, 1984, Nucleic Acids Research

15 12:387). Examples of other software than can perform sequence comparisons include, but are not limited to, the BLAST package (see Ausubel *et al.*, 1999 *ibid* – Chapter 18), FASTA (Atschul *et al.*, 1990, J. Mol. Biol., 403-410) and the GENEWORKS suite of comparison tools. Both BLAST and FASTA are available for offline and online searching (see Ausubel *et al.*, 1999 *ibid*, pages 7-58 to 7-60). However, for

20 some applications, it is preferred to use the GCG Bestfit program. A new tool, called BLAST 2 Sequences is also available for comparing protein and nucleotide sequence (see FEMS Microbiol Lett 1999 174(2): 247-50; FEMS Microbiol Lett 1999 177(1): 187-8 and tatiana@ncbi.nlm.nih.gov).

25 Although the final % homology can be measured in terms of identity, the alignment process itself is typically not based on an all-or-nothing pair comparison. Instead, a scaled similarity score matrix is generally used that assigns scores to each pairwise comparison based on chemical similarity or evolutionary distance. An example of such a matrix commonly used is the BLOSUM62 matrix - the default matrix for the

30 BLAST suite of programs. GCG Wisconsin programs generally use either the public default values or a custom symbol comparison table if supplied (see user manual for further details). For some applications, it is preferred to use the public default values

for the GCG package, or in the case of other software, the default matrix, such as BLOSUM62.

Once the software has produced an optimal alignment, it is possible to calculate %
 5 homology, preferably % sequence identity. The software typically does this as part of the sequence comparison and generates a numerical result.

The sequences may also have deletions, insertions or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent
 10 substance. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues as long as the secondary binding activity of the substance is retained. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino
 15 acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine, valine, glycine, alanine, asparagine, glutamine, serine, threonine, phenylalanine, and tyrosine.

Conservative substitutions may be made, for example according to the Table below.
 20 Amino acids in the same block in the second column and preferably in the same line in the third column may be substituted for each other:

ALIPHATIC	Non-polar	G A P
		I L V
	Polar - uncharged	C S T M
		N Q
	Polar - charged	D E
		K R
AROMATIC		H F W Y

The present invention also encompasses homologous substitution (substitution and
 25 replacement are both used herein to mean the interchange of an existing amino acid

residue, with an alternative residue) may occur i.e. like-for-like substitution such as basic for basic, acidic for acidic, polar for polar etc. Non-homologous substitution may also occur i.e. from one class of residue to another or alternatively involving the inclusion of unnatural amino acids such as ornithine (hereinafter referred to as Z),
 5 diaminobutyric acid ornithine (hereinafter referred to as B), norleucine ornithine (hereinafter referred to as O), pyriylalanine, thienylalanine, naphthylalanine and phenylglycine.

Replacements may also be made by unnatural amino acids include; alpha* and alpha-disubstituted* amino acids, N-alkyl amino acids*, lactic acid*, halide derivatives of
 10 natural amino acids such as trifluorotyrosine*, p-Cl-phenylalanine*, p-Br-phenylalanine*, p-I-phenylalanine*, L-allyl-glycine*, β -alanine*, L- α -amino butyric acid*, L- γ -amino butyric acid*, L- α -amino isobutyric acid*, L- ϵ -amino caproic acid[#], 7-amino heptanoic acid*, L-methionine sulfone^{#*}, L-norleucine*, L-norvaline*, p-nitro-L-phenylalanine*, L-hydroxyproline[#], L-thioprolin*, methyl derivatives of
 15 phenylalanine (Phe) such as 4-methyl-Phe*, pentamethyl-Phe*, L-Phe (4-amino)[#], L-Tyr (methyl)*, L-Phe (4-isopropyl)*, L-Tic (1,2,3,4-tetrahydroisoquinoline-3-carboxyl acid)*, L-diaminopropionic acid [#] and L-Phe (4-benzyl)*. The notation * has been utilised for the purpose of the discussion above (relating to homologous or
 20 non-homologous substitution), to indicate the hydrophobic nature of the derivative whereas # has been utilised to indicate the hydrophilic nature of the derivative, #* indicates amphipathic characteristics.

Variant amino acid sequences may include suitable spacer groups that may be inserted
 25 between any two amino acid residues of the sequence including alkyl groups such as methyl, ethyl or propyl groups in addition to amino acid spacers such as glycine or β -alanine residues. A further form of variation, involves the presence of one or more amino acid residues in peptoid form, will be well understood by those skilled in the art. For the avoidance of doubt, "the peptoid form" is used to refer to variant amino
 30 acid residues wherein the α -carbon substituent group is on the residue's nitrogen atom rather than the α -carbon. Processes for preparing peptides in the peptoid form are

known in the art, for example Simon RJ et al., PNAS (1992) 89(20), 9367-9371 and Horwell DC, Trends Biotechnol. (1995) 13(4), 132-134.

The term “fragment” indicates that the polypeptide comprises a fraction of the wild-type amino acid sequence. It may comprise one or more large contiguous sections of sequence or a plurality of small sections. The polypeptide may also comprise other elements of sequence, for example, it may be a fusion protein with another protein. Preferably the polypeptide comprises at least 50%, more preferably at least 65%, most preferably at least 80% of the wild-type sequence.

With respect to function, the mutant, variant, homologue or fragment should be capable of transducing adipose tissue when used to pseudotype an appropriate vector.

The viral delivery system used in the present invention may comprise nucleotide sequences that can hybridise to the nucleotide sequence presented herein (including complementary sequences of those presented herein). In a preferred aspect, the present invention covers nucleotide sequences that can hybridise to the nucleotide sequence of the present invention under stringent conditions (e.g. 65°C and 0.1 SSC) to the nucleotide sequence presented herein (including complementary sequences of those presented herein).

A potential advantage of using the rabies glycoprotein in comparison to the VSV glycoprotein is the detailed knowledge of its toxicity to man and other animals due to the extensive use of rabies vaccines. In particular phase 1 clinical trials have been reported on the use of rabies glycoprotein expressed from a canarypox recombinant virus as a human vaccine (Fries *et al.*, 1996 Vaccine 14, 428-434), these studies concluded that the vaccine was safe for use in humans.

ADIPOSE TISSUE

As used herein, the term “adipose tissue” and particularly “white adipose tissue” means a highly specialised tissue developed particularly in mammals and birds to store fat to supply energy to the whole animal. The cells contain a single large droplet

of fat, that in the nourished state, fills most of the cell. The nucleus is somewhat flattened and is in the narrow band of cytoplasm around the cell's periphery. Adipose tissue is found in many parts of the body but tends to be concentrated under the skin (subcutaneous) and around internal organs (heart and kidney). The stored fat consists
5 principally of triacylglycerols.

Another type of adipose tissue, called "brown adipose tissue", which is a highly specialised tissue with a high content of lipid and cytochromes, is found in some animals, particularly hibernating animals and the newborn of some species. It is
10 highly vascular and consists of small polygonal cells, each containing many separate lipid droplets and many mitochondria. Its function is thermogenesis during the arousal period after hibernation or, in the young, to provide heat before shivering has developed. It is active, also, in normal but not in obese humans. The colour is due to the high cytochrome content. Heat is generated by lipid oxidation through electron
15 transport not coupled to oxidative phosphorylation. The uncoupling is mediated by brown fat uncoupling protein.

Adipose tissue is a large easily accessible tissues that is well vascularized. Thus, the delivery of an NOI to adipose tissue allows the rapid release of therapeutic proteins
20 into the circulation. Adipose tissue cells are also long lived and non-dividing, thus, allowing long term expression of one of more proteins of interest (POIs) encoded by an nucleotide sequence of interest (NOI).

NOIs

25

In the present invention one or more NOIs (nucleotide sequences of interest) may be delivered to a target cell *in vivo* or *in vitro*.

In accordance with the present invention, it is possible to manipulate the viral genome
30 so that viral genes are replaced or supplemented with one or more NOIs which may be heterologous NOIs.

The term "heterologous" refers to a nucleic acid or protein sequence linked to a nucleic acid or protein sequence to which it is not naturally linked.

In the present invention, the term NOI includes any suitable nucleotide sequence, which need not necessarily be a complete naturally occurring DNA or RNA sequence. Thus, the NOI can be, for example, a synthetic RNA/DNA sequence, a recombinant RNA/DNA sequence (i.e. prepared by use of recombinant DNA techniques), a cDNA
5 sequence or a partial genomic DNA sequence, including combinations thereof. The sequence need not be a coding region. If it is a coding region, it need not be an entire coding region. In addition, the RNA/DNA sequence can be in a sense orientation or in an anti-sense orientation. Preferably, it is in a sense orientation. Preferably, the sequence is, comprises, or is transcribed from cDNA.

10

In one embodiment of the present invention, a lentiviral vector system comprises at least one NOI.

A lentiviral vector genome may generally comprise LTRs at the 5' and 3' ends,
15 suitable insertion sites for inserting one or more NOI(s), and/or a packaging signal to enable the genome to be packaged into a vector particle in a producer cell. There may even be suitable primer binding sites and integration sites to allow reverse transcription of the vector RNA to DNA, and integration of the proviral DNA into the target cell genome. In a preferred embodiment, the lentiviral vector particle has a
20 reverse transcription system (compatible reverse transcription and primer binding sites) and an integration system (compatible integrase and integration sites).

The NOI may encode a protein of interest ("POI"). In this way, a lentiviral delivery system could be used to examine the effect of expression of a foreign gene a target
25 adipose tissue cell. For example, a lentiviral delivery system could be used to screen a cDNA library for a particular effect on a target adipose tissue site.

The NOI may be capable of blocking or inhibiting the expression of a gene in the target adipose tissue site. For example, the NOI may be an antisense sequence. The
30 inhibition of gene expression using antisense technology is well known.

The NOI or a sequence derived therefrom may be capable of "knocking out" the expression of a particular gene in a target adipose tissue site. There are several "knock out" strategies known in the art. For example, the NOI may be capable of
5 integrating in the genome of the cells of the target adipose tissue site so as to disrupt expression of the particular gene. The NOI may disrupt expression by, for example, introducing a premature stop codon, by rendering the downstream coding sequence out of frame, or by affecting the capacity of the encoded protein to fold (thereby affecting its function).

10

Alternatively, the NOI may be capable of enhancing or inducing ectopic expression of a gene in the target adipose tissue site. The NOI or a sequence derived therefrom may be capable of "knocking in" the expression of a particular gene.

15 Transduced adipose tissue cells which express a particular gene, or which lack the expression of a particular gene have applications in drug discovery and target validation. The expression system could be used to determine which genes have a desirable effect on target adipose tissue cells, such as those genes or proteins which are able to prevent or reverse the triggering of secretory activity in the adipose tissue
20 cells. Equally, if the inhibition or blocking of expression of a particular gene is found to have an undesirable effect on the target adipose tissue cells, this may open up possible therapeutic strategies which ensure that expression of the gene is not lost.

An NOI delivered by the viral delivery system of the present invention, such as a
25 lentiviral vector delivery system, may be a selection gene, or a marker gene. Many different selectable markers have been used successfully in retroviral vectors. These are reviewed in "Retroviruses" (1997 Cold Spring Harbour Laboratory Press Eds: JM Coffin, SM Hughes, HE Varmus pp 444) and include, but are not limited to, the bacterial neomycin and hygromycin phosphotransferase genes which confer resistance
30 to G418 and hygromycin respectively; a mutant mouse dihydrofolate reductase gene which confers resistance to methotrexate; the bacterial *gpt* gene which allows cells to grow in medium containing mycophenolic acid, xanthine and aminopterin; the bacterial *hisD* gene which allows cells to grow in medium without histidine but

containing histidinol; the multidrug resistance gene (*mdr*) which confers resistance to a variety of drugs; and the bacterial genes which confer resistance to puromycin or phleomycin. All of these markers are dominant selectable and allow chemical selection of most cells expressing these genes.

5

An NOI delivered by a viral delivery system of the present invention, such as a lentiviral delivery system may be a therapeutic gene - in the sense that the gene itself may be capable of eliciting a therapeutic effect or it may code for a product that is capable of eliciting a therapeutic effect.

10

In one preferred embodiment, the NOI is capable of encoding a factor that regulates whole body metabolism.

Preferably the NOI encodes a factor that regulates body weight and lipid metabolism.

15

Preferably the NOI encodes a factor that regulates fat and glucose metabolism.

Preferably the NOI encodes an adipocyte complement related protein (see Fruebis et al PNAS (2001) 98: 2005-2010).

20

Preferably the NOI encodes a gene associated with a blood disorder, such as an hereditary blood disorder, including a gene associated with the treatment of such a disease.

25 Preferably the NOI encodes a blood factor such as but not limited to erythropoietin (EPO), Factor VIII or Factor IX.

Preferably the NOI encodes a gene associated with a vascular disease including a gene associated with the treatment of such a disease such as but not limited to angiostatic factors, VEGF, nitric-oxide synthase, thymidine kinase, retinoblastoma, anti-apoptotic molecules, growth arrest homoeobox, tissue inhibitor of metalloproteinases, cyclin or
30 cyclin-dependent kinase inhibitors, fas ligand and hirudin, and antisense oligonucleotides against transcription factors or cell-cycle regulatory proteins.

Preferably the NOI encodes an uncoupling protein (Kozak (2000) 6: 1092-1093).

5 In accordance with the present invention, suitable NOIs include those that are of therapeutic and/or diagnostic application such as, but not limited to: sequences encoding cytokines, chemokines, hormones, antibodies, anti-oxidant molecules, engineered immunoglobulin-like molecules, a single chain antibody, fusion proteins, enzymes, immune co-stimulatory molecules, immunomodulatory molecules, anti-sense RNA, a transdominant negative mutant of a target protein, a toxin, a conditional
10 toxin, an antigen, a tumour suppresser protein and growth factors, membrane proteins, vasoactive proteins and peptides, anti-viral proteins and ribozymes, and derivatives thereof (such as with an associated reporter group). The NOIs may also encode pro-drug activating enzymes.

15 As used herein, "antibody" includes a whole immunoglobulin molecule or a part thereof or a bioisostere or a mimetic thereof or a derivative thereof or a combination thereof. Examples of a part thereof include: Fab, F(ab)'₂, and Fv. Examples of a bioisostere include single chain Fv (ScFv) fragments, chimeric antibodies, bifunctional antibodies.

20 The term "mimetic" relates to any chemical which may be a peptide, polypeptide, antibody or other organic chemical which has the same binding specificity as the antibody.

25 The term "derivative" as used herein includes chemical modification of an antibody. Illustrative of such modifications would be replacement of hydrogen by an alkyl, acyl, or amino group.

SCREENING METHODS

30 In further aspect the present invention also relates to a screening method and modulating factors isolatable by such methods, and uses for such factors.

In one embodiment, the present invention provides method for screening for modulating factors for adipose tissue cells, which comprises the following steps:

- (i) providing a target adipose tissue cell;
- (ii) transducing the adipose tissue cell with a cDNA library capable of encoding a plurality of candidate compounds;
- (ii) monitoring the expression of the candidate compounds; and
- (iii) screening for a candidate compound capable of modulating an activity of the target adipose tissue cell.

The adipose tissue cells may be transduced using a lentiviral vector system.

The cDNA library may be a ribozyme library constructed in a lentiviral vector. The ribozyme library can include but is not limited to a hammerhead ribozyme, an EGS or a group II intron ribozyme. The ribozyme library may be used to transduce cell types of interest *in vitro* and *in vivo*. These cells can then be screened for the phenotype of interest. The gene or genes affected by the ribozyme can be elucidated by PCR analysis of the ribozyme. For general teachings on ribozymes, see WO 99/41397.

The advantage of doing this with a lentivector is that it allows the transduction of primary (non-dividing) cells.

In another embodiment, the present invention provides a modulating factor for adipose tissue cells identifiable by the method as described above.

PHARMACEUTICAL COMPOSITIONS

The present invention also provides the use of a viral delivery system as defined in the first aspect of the invention in the manufacture of a pharmaceutical composition. The

pharmaceutical composition may be used to deliver a NOI to a target adipose tissue cell in need of same.

5 The pharmaceutical composition may be used for treating an individual by gene therapy, wherein the composition comprises or is capable of producing a therapeutically effective amount of a viral vector system according to the present invention.

10 The method and pharmaceutical composition of the invention may be used to treat a human or animal subject. Preferably the subject is a mammalian subject. More preferably the subject is a human. Typically, a physician will determine the actual dosage which will be most suitable for an individual subject and it will vary with the age, weight and response of the particular patient.

15 The composition may optionally comprise a pharmaceutically acceptable carrier, diluent, excipient or adjuvant. The choice of pharmaceutical carrier, excipient or diluent can be selected with regard to the intended route of administration and standard pharmaceutical practice. The pharmaceutical compositions may comprise as (or in addition to) the carrier, excipient or diluent, any suitable binder(s), lubricant(s),
20 suspending agent(s), coating agent(s), solubilising agent(s), and other carrier agents that may aid or increase the viral entry into the target adipose tissue site (such as for example a lipid delivery system).

25 Where appropriate, the pharmaceutical compositions can be administered by any one or more of: inhalation, in the form of a suppository or pessary, topically in the form of a lotion, solution, cream, ointment or dusting powder, by use of a skin patch, orally in the form of tablets containing excipients such as starch or lactose, or in capsules or ovules either alone or in admixture with excipients, or in the form of elixirs, solutions or suspensions containing flavouring or colouring agents, or they can be injected
30 parenterally, for example intracavernosally, intravenously, intramuscularly or subcutaneously. For parenteral administration, the compositions may be best used in the form of a sterile aqueous solution which may contain other substances, for example enough salts or monosaccharides to make the solution isotonic with blood.

For buccal or sublingual administration the compositions may be administered in the form of tablets or lozenges which can be formulated in a conventional manner.

The vector system used in the present invention may conveniently be administered by
5 direct injection into the patient.

DISEASES

The viral vector system used in the present invention is particularly useful in treating
10 and/or preventing a disease which is associated with the death or impaired function of adipose tissue cells.

In particular, the vector system used in the present invention may be used to treat and
/or prevent a disease which is associated with a derangement in the metabolism of
15 adipose tissue cells.

In particular the present invention may be used to treat and/or prevent a condition associated with obesity, diabetes and/or whole body metabolism.

20 The viral vector system used in the present invention is particularly useful in treating and/or preventing a disease which is associated hereditary blood disorders.

The viral vector system used in the present invention is particularly useful in treating and/or preventing vascular diseases.
25

The invention will now be further described by way of Examples, which are meant to serve to assist one of ordinary skill in the art in carrying out the invention and are not intended in any way to limit the scope of the invention. The Examples refer to the Figures. In the Figures:
30

Figure 1 shows a photographic representation;

Figure 2 shows a photographic representation;

Figure 3 shows a photographic representation;

Figure 4 shows a photographic representation;

5 Figure 5 shows a photographic representation;

Figure 6 shows a photographic representation;

Figure 7 shows a photographic representation;

10

Figure 8 shows a plasmid map of pONY8.0Z;

Figure 9 shows a plasmid map of pONY8.0G;

15 Figure 10 shows a plasmid map of pONY8.0Z5'pos del CTS;

Figure 11 shows a schematic representation;

Figure 12 shows features of pSmart 2 MCS 5'cppt;

20

Figure 13 shows a graphical plot; and

Figure 14 shows a graphical plot.

25 In more detail:

Figure 1 shows β -galactosidase expression in the mammary fat pad tissue of nude mice, transduced with EIAV pONY8.0Z 5' pos del CTS vector, 14 days post injection;

30

Figure 2 shows β -galactosidase expression in rat gastrocnemius muscle. 14 days after the injection of VSV-G pseudotyped pONY8.0Z 5' pos del CTS EIAV vector;

Figure 3 shows β -galactosidase expression in mouse gastrocnemius muscle. 14 days after the injection of VSV-G pseudotyped pONY8.0Z 5' pos del CTS EIAV vector;

Figure 4 shows β -galactosidase expression in mouse gastrocnemius muscle. 28 days after the injection of VSV-G pseudotyped pONY8.0Z 5' pos del CTS EIAV vector;

Figure 5 shows β -galactosidase expression in rat gastrocnemius muscle. 28 days after the injection of VSV-G pseudotyped pONY8.0Zcppt EIAV vector;

Figure 6 shows β -galactosidase expression in mouse gastrocnemius muscle. 84 days after the injection of VSV-G pseudotyped pONY8.0Z 5' pos del CTS EIAV vector;

Figure 7 shows β -galactosidase expression in rat gastrocnemius muscle. 84 days after the injection of VSV-G pseudotyped pONY8.0Z 5' pos del CTS EIAV vector;

Figure 13 shows the amount of factor IX produced by D17 cells transduced with either an EIAV vector coding for factor IX (Smart2FIX) or β -galactosidase (Smart 2Z) or infected with an adenovirus vector expressing factor IX (Ad FIX); and

Figure 14 shows the bleeding tendency 24 hours after injecting EIAV factor IX encoding genome DNA into the tail vein of a mouse model of haemophilia.

EXAMPLES

Plasmid construction:

a) Vector plasmids

Numbering used is as of Payne *et al* 1994 (J. Gen Virol. 75:425-429). The pONY series of vectors and their pseudotyping with the different envelopes have been described previously (WO99/61639) (Mitrophanous *et al.*1999 Gene Ther 1999 6:1808-1818). pONY8.0Z (Figure 8, SEQ ID No 1) was derived from pONY4.0Z (WO99/32646) by introducing mutations which prevented expression of TAT by an 83nt deletion in the exon 2 of tat, prevented S2 expression by a 51nt deletion, prevented REV expression by deletion of a single base within exon 1 of rev and

prevented expression of the N-terminal portion of gag by insertion of T in the first two ATG codons of gag, thereby changing the sequence to ATTG from ATG. With respect to the wild type EIAV sequence (Acc. No. U01866) these correspond to deletion of nt 5234-5316 inclusive, nt 5346-5396 inclusive and nt 5538. The insertion of T residues was after nt 526 and 543. pONY8.0G (Figure 9, SEQ ID No 2) was derived from pONY8.0Z by exchange of the Lac Z reporter gene for the enhanced green fluorescent protein (GFP) gene. This was done by transferring the *Sac* II – *Kpn* I fragment corresponding to the GFP gene and flanking sequences from pONY4.0G (WO99/32646) into pONY8.0Z cut with the same enzymes.

Construction of pONY8.0Z 5'POS del CTS

The presence of a sequence termed the central polypurine tract and central termination sequence (cPPT-see Stetor *et al.* Biochemistry. 1999 Mar 23;38(12):3656-6) improves the efficiency of gene delivery to non-dividing cells (WO 99/55892). This *cis*-acting element is located in the polymerase coding region element and can be obtained as a functional element by using PCR amplification using any plasmid which contains the EIAV polymerase coding region (for example pONY3.1) as follows. The PCR product includes the central polypurine tract and the central termination sequence (CTS). The oligonucleotide primers used in the PCR reaction were:

EIAV cPPT POS: CAGGTTATTCTAGAGTCGACGCTCTCATTACTTGTAAC

EIAV cPPT NEG: CGAATGCGTTCTAGAGTCGACCATGTTCCACCAGGGATTTTG

Recognition sequences for *Xba*I are shown in *italic* and use of this enzyme facilitates insertion of the PCR product into the pONY8.0Z backbone.

Before insertion of the cPPT/CTS PCR product prepared as described above, the vector backbone was modified to remove the CTS which is already present due the presence of some EIAV *pol* sequences downstream of the reporter gene. This was achieved by subcloning the *Sal*II to *Sca*I fragment encompassing the CTS and RRE

region from pONY8.0Z into pSP72 (Genbank Acc.No.X65332), prepared for ligation by digestion with *SalI* and *EcoRV*. The CTS region was then removed by digestion with *KpnI* and *PpuMI*, the overhanging ends ‘blunted’ by T4 DNA polymerase treatment and then the ends religated. The modified EIAV vector fragment was then excised using *SalI* and *NheI* and ligated into pONY8.0Z prepared for ligation by digestion with the same enzymes. This new EIAV vector was termed pONY8.0Z delCTS.

pONY8.0Z delCTS has a unique *XbaI* site which is located immediately upstream of the CMV-LacZ unit. The cPPT/CTS PCR product was digested with *XbaI* and then ligated into pONY8.0Z delCTS prepared for ligation by digestion with *XbaI*. Ligation into this site results in plasmids with the cPPT/CTS element in either the positive or negative senses. A clone in which the cPPT/CTS was in the positive sense (functionally active) and located to the 5’-side of the internal CMV promoter was selected and termed pONY8.0Z 5’POS delCTS (SEQ ID No 3 and Figure 10) The cPPT/CTS sequence was also inserted into pONY8.0Z delCTS, downstream of the CMV-LacZ reporter by using the unique *SalI* site present in the plasmid. To achieve this the cPPT/CTS PCR primer was digested with *SalI* and then ligated with pONY8.0Z delCTS prepared for ligation by digestion with *SalI*. A plasmid in which the cPPT/CTS was in the positive sense was selected and termed pONY8.0Z 3’POS delCTS.

Construction of Smart2Fix and Smart2Z

A human factor IX gene (FIX) and a β -galactosidase gene were introduced into a pSmart2 5’cppt backbone (Figure 13 and SEQ ID NO 4) to produce Smart2Fix and Smart2Z respectively. The plasmid pSmart2 is a derivative of pONY8.0Z which has been altered to contain fewer of the nucleotides derived from the EIAV envelope region, to contain the central polypurine tract and the Woodchuck hepatitis post-transcriptional regulatory element, and to have alterations carried out to the 3’ LTR to make the vector self inactivating. Vector production was performed using the three plasmid co-transfection procedure to yield a product with an equivalent number of

RNA genome copies to the β -galactosidase encoding, control vector, with a biological end point titre of over 10^9 tu per ml.

5 b) Envelope plasmids

pSA91ERAwT was used for pseudotyping with rabies G. This plasmid has been described previously (WO99/61639) under the name “pSA91RbG”. Briefly, pSA91ERAwT was constructed by cloning 1.7 kbp *Bgl*II rabies G fragment (strain ERA) from pSG5rabgp (Burger *et al.*, 1991 J.Gen. Virol. 72. 359-367) into pSA91, a
10 derivative of pGW1HG (Soneoka *et al* 1995 Nucl. Acids Res. 23: 628-633) from which the *gpt* gene has been removed by digestion with *Bam*HI and re-ligation. This construct, pSA91ERAwT, allows expression of rabies G from the human cytomegalovirus (HCMV) immediate early gene promoter-enhancer.

15 pRV67 was used for pseudotyping with VSV-G. pRV67 (described in WO99/61639) is a VSV-G expression plasmid in which VSV-G was expressed under the control of human cytomegalovirus promoter/enhancer, in place of rabies G in pSA91ERAwT.

Production and Assay of Vectors: Vector stocks were generated by calcium-phosphate
20 transfection of human kidney 293T cells plated on 10 cm dishes with 16 μ g of vector plasmid, 16 μ g of gag/pol plasmid and 8 μ g of Rabies G envelope plasmid. 36-48 h after transfection, supernatants were filtered (0.45 μ m) aliquoted and stored at -70°C. Concentrated vector preparations were made by initial low speed centrifugation 6 000 x g (JLA-10.500 for 16 hours at 4 °C followed by ultracentrifugation at 20 000 rpm
25 (SW40Ti rotor) for 90 min, at 4 °C. The virus was resuspended in PBS for 3-4 h aliquoted and stored at -70 °C. Transduction was carried out in the presence of polybrene (8 μ g/ml).

30

Gene transfer with EIAV vectors into adipose tissue and muscle cells.

Transduction of adipose tissue.

40 μ l of VSV-G pseudotyped EIAV pONY8.0Z 5' pos del CTS vector containing 4×10^7 TU was injected into the mammary fat pads of six nude mice and into the fatty tissue near to the epididymius of six nude rats. Controls of three mice and three rats were used where 40 μ l of the buffer used to re-suspend the virus was injected into the appropriate sites. Two mice and two rats were culled on days 14, 28 and 84. A single control animal was culled on each day. The tissues were harvested and snap frozen in liquid nitrogen. These were then sectioned and stained for β -galactosidase activity.

Significant staining was observed in the mouse mammary fat pad samples throughout the duration of the experiment. Images of sections taken after 14 days after the vectors were injected are shown in figure 1. No staining was observed in any of the rats.

Transduction of skeletal muscle.

10 μ l aliquots of VSV-G pseudotyped EIAV pONY8.0Z 5' pos del CTS vector was injected into four different sites in the gastrocnemius muscle of the left legs of six nude mice and six nude rats. This is a dose of 4×10^7 TU per animal. Controls of three mice and three rats were used where 10 μ l aliquots of the buffer used to re-suspend the virus were injected into the appropriate sites. Two mice and two rats were culled on days 14, 28 and 84. A single control animal was culled on each day. The tissues were harvested and snap frozen in liquid nitrogen. These were then sectioned and stained for β -galactosidase activity.

Significant staining was observed in all of the test mice examined and in 5 out of the six rats examined.

Gene transfer with EIAV vectors into adipose cells for treatment of blood disorders

A minimal lentivector (such as EIAV, HIV and FIV), preferably an EIAV vector derived from the previously described pONY8.0Z, is engineered to encode for a gene involved with a hereditary blood disorder. Such a vector is used to transduce adipose tissues. These cells, which have a good connection to the circulatory system, secrete the protein of choice into the local circulatory system, which should result in an improvement of the disease condition. The nucleotide sequence of the selected gene

may be altered so as to optimize the codon usage in such a way as to increase gene expression or to increase RNA stability or both. This is performed without altering the amino acid coding sequence.

5 Depending on the therapeutic protein of choice, the amount of protein delivered is controlled by the number of cells transduced or by the use of promoters whose activity may be regulated. Regulation and or increased gene transfer efficiency may also be achieved by the removal of adipose cells from the patient followed by ex vivo transduction and possibly expansion before implantation of the altered cells in the
10 patient. Such implantation may involve the cells being reintroduced in an encapsulated form; this may allow easy cessation of therapy if the treatment results in any adverse effects. The site of injection may be dependent on the disease to be treated.

15 Adipose cells are found in the vicinity of the salivary glands. The anatomy of these glands has found to be altered by leptin, a hormone which may be secreted by adipose cells as well as a range of other cells. It is possible to target foreign proteins into the saliva using these cells as factories for foreign proteins. Since these tissues are highly vascularised, it is expected that any foreign proteins expressed in these cells is
20 secreted into the blood system.

There is evidence to suggest that adipocytes in the minor deposits that enclose lymph nodes may be specialized to supply immune cells with the fuel and materials they need to mount a prompt, effective response to foreign invasion (Pond (2000) Biologist
25 (London) June; 47(3):147-150). Recruitment of additional adipocytes to the lymph nodes during the early stages of an immune response has been reported (Pond and Mattacks (2002) Cytokine Feb; 17 (3):131-139). It may be possible to use these adipocytes to modulate immune responses.

30 Factor IX gene transfer

D17 cells were transduced with an ElAV vector coding for factor IX (pSmart2FIX) or control β -galactosidase (pSmart2Z) or infected with an adenovirus vector expressing

factor IX. The amount of factor IX in the supernatant was measured by ELISA 24 hours after transduction.

At least as much factor IX was produced by the EIAV vector encoding factor IX as
5 the adenovirus vector encoding factor IX when normalised to multiplicity of infection (Figure 13). As expected factor IX was not produced by cells transduced with the control, β -galactosidase encoding vector.

EIAV factor encoding genome DNA was injected into the tail vein of a mouse model
10 of haemophilia. Vector genome efficiency was assessed 24 hours after injection by measuring the bleeding tendency of the mice treated with factor IX in comparison with untreated mice and normal mice. The data shown in Figure 14 shows that enough factor protein was expressed from the pSmart2FIX genome to have an effect on the length of time that the treated mice bleed for after injury. Since an effect was seen
15 when vector genome DNA was injected into mice, experiments will be performed using the vector particles.

Gene transfer of a factor VIII derivative

20 A minimal EIAV vector derived from pONY8.0Z is used to express factor VIII a derivative such as with a deletion in the B-domain or a similar deletion. Many different forms of factor VIII with deletions in the B-domain which retain some degree of functionality are described in the literature. Eaton et al (1986) Biochemistry Dec 30; 25 (26):8343-8347 described a polypeptide in which amino acids 797 to 1562
25 are deleted. This sequence has been used to construct an MLV-based retroviral by Israel and Kaufman (1990) Blood Mar 1; 75 (5):1074-1080. Pittman et al. (1993) Blood Jun 1; 81 (11):2925-2935 describe a form of factor VIII in which the B-domain amino acid sequences have been deleted from 760 to 1639 and which has biochemical properties similar to those of full length factor VIII. This sequence deletion is
30 described in patent WO 86/06101. US 6,346,513 B1 describes a series of derivatives of factor VIII which possess activity. The deletions have two benefits as regards the

production of minimal EIAV vectors. Firstly the encoding sequence for full-length protein is present on a 7440bp SalI-HpaI fragment which is approaching the maximum size limit for the EIAV vectors. Secondly the deleted forms of the protein are reported to be expressed at significantly higher levels in a range of different expression systems than the wild type. A minimal EIAV vector is constructed by cloning the sequence for a B-domain deleted form of the protein into the vector genome. A factor VIII knockout mouse model for haemophilia A exists (Bi et al 1995 Nat. Genet. May; 10 (1):119-121). Adipose tissues in this mouse are transduced with minimal EIAV vector encoding factor VIII and serum levels of factor VIII are assessed using commercially available reagents (eg. An ELISA to factor VIII is supplied by AbCam: Factor VIII RFFVIIIIC/8 - ab6345). If levels indicate that expression has occurred bleeding tendency after tail clipping is assessed. If efficacy is observed in the mouse model further investigations with a dog model are conducted.

15

Gene transfer of human insulin

The human proinsulin gene is inserted into a minimal EIAV vector. This vector is used to transduce adipocytes. The nature of the disease is such that the expression of proinsulin in any un-encapsulated transduced adipose cells has to be regulated. A variety of ways of regulating insulin expression have been described. For instance patent US 6,352,857 (Treatment of diabetes with synthetic beta cells) described a method for obtaining glucose-regulated expression of active insulin in the cells of a mammalian subject. The method involves the use of glucose inducible regulatory elements in a promoter suitable for expression in the cells of choice coupled with an amino acid alteration in the coding sequence to ensure correct processing of proinsulin to insulin in the producer cells. A suicide gene such as HSV thymidine kinase may be encoded by the vector, allowing for the rapid removal of transduced cells from the patient using ganciclovir if harmful side effects result from the treatment.

30

Gene transfer of angiostatic factors

The stable and localized production of angiostatic factors near to sites of increased vascularisation may be a more effective way of treatment than either the systemic injection of such factors or local injection. Factors expressed by adipocytes are readily secreted into the blood and some of the factors normally expressed by adipocytes such as proteins of the renin-angiotensin system may have significant effects on blood pressure and cardiovascular responses especially in obese individuals.

Angiogenesis is an essential component of tumour growth and metastasis. The production of angiostatic factors by transduced adipose cells in the vicinity of a tumour into the local blood vessels may prevent tumour angiogenesis. The transduction of the larger adipose tissues such as are found around the waist, flank, buttocks and abdomen allows anti-angiogenic factors to be delivered at therapeutic concentrations systemically for instance to prevent or reduce the growth rates of metastases. Minimal EIAV vectors may be used to express proteins such as thrombospondin or the type I repeat peptides described in WO 00/47622 to inhibit the proliferation and migration of blood vessel endothelial cells.

Other vascular diseases may be treated with the local expression of therapeutic factors from adipose cells localized around disease tissues. Promising therapeutic effects have been obtained in animal models of restenosis or vein-graft thickening with the transfer of genes coding for VEGF, nitric-oxide synthase, thymidine kinase, retinoblastoma, growth arrest homoeobox, tissue inhibitor of metalloproteinases, cyclin or cyclin-dependent kinase inhibitors, fas ligand and hirudin, and antisense oligonucleotides against transcription factors or cell-cycle regulatory proteins.

Discussion

The results demonstrate that long term expression may be maintained in adipose and skeletal muscle cells using minimal EIAV vectors.

Both adipose tissue and skeletal muscle tissue have features that make them suitable to use as sites to manufacture therapeutically useful proteins. By way of example, adipose tissue has a major endocrine role in addition to its triglyceride storage role. The most important endocrine role of adipose tissue is probably the secretion of leptin. However other factors such as sex steroids and glucocorticoids, peptide hormone precursors (eg angiotensinogen), complement factors, pro-inflammatory cytokines, interleukin 6, transforming growth factor-beta, tissue factor, plasminogen activator inhibitor-1 Aipo Q and adiponectin are also secreted. Aberrant function of this tissue is associated with diseases such as diabetes (related to low levels of leptin secretion). Moreover, adipose cells have a good connection to the circulatory system and are found at many sites around the human body, for instance in mammary tissue, around lymph nodes, kidneys and salivary glands, around the waist, flank, thigh, buttocks and in the abdomen. This means that the use of adipose deposits near to a target organ for therapeutic protein expression allows the treatment of a wide range of diseases. Thus the regulation or alteration of the secretory functions of adipose cells by gene delivery may be used to treat a varied range of diseases.

Consequently, the use of a viral vector system, such as a lentiviral vector system (such as EIAV and HIV lentiviral vectors systems) may be used to facilitate studies into the ways in which adipose tissue secretions are involved in disease phenotypes. These vectors may be used to selectively over or under express identified targets or to introduce cDNA or ribozyme libraries to identify new targets for pharmaceutical or gene delivery treatments.

All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention

which are obvious to those skilled in chemistry, biology or related fields are intended to be within the scope of the following claims.

SEQUENCE LISTINGS

SEQ ID NO 1

5 PONY8.0Z

AGATCTTGAATAATAAAATGTGTGTTTGTCCGAAATACGCGTTTTGAGATTCTGTGCGCC
 GACTAAATTCATGTGCGCGGATAGTGGTGTATCGCCGATAGAGATGGCGATATTGGAA
 AAATTGATATTTGAAAATATGGCATATTGAAAATGTGCGCCGATGTGAGTTTCTGTGTAAC
 10 TGATATCGCCATTTTCCAAAAGTGATTTTGGGCATACGCGATATCTGGCGATAGCGCT
 TATATCGTTTACGGGGGATGGCGATAGACGACTTTGGTGACTTGGGCGATTCTGTGTGTC
 GCAAATATCGCAGTTTCGATATAGGTGACAGACGATATGAGGCTATATCGCCGATAGAGG
 CGACATCAAGCTGGCACATGGCCAATGCATATCGATCTATACATTGAATCAATATTGGCC
 ATTAGCCATATTATTGATTGGTTATATAGCATAAATCAATATTGGCTATTGGCCATTGCA
 15 TACGTTGTATCCATATCGTAATATGTACATTTATATTGGCTCATGTCCAACATTACCGCC
 ATGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCA
 TAGCCCATATATGGAGTTCGCGTTACATAAATTACGGTAAATGGCCCGCTGGCTGACC
 GCCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAAT
 AGGGACTTTCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGT
 20 ACATCAAGTGTATCATATGCCAAGTCCGCCCCCTATTGACGTCAATGACGGTAAATGGCC
 CGCTTGGCATTATGCCCAGTACATGACCTTACGGGACTTTCCTACTTGGCAGTACATCTA
 CGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACACCAATGGGCGTGG
 ATAGCGGTTTGACTCAGGGGATTTCCAAGTCTCCACCCATTGACGTCAATGGGAGTTT
 GTTTTGGCACAAAATCAACGGGACTTTCAAAATGTCGTAACAACCTGCGATCGCCCGCC
 25 CCGTTGACGCAATGGGCGGTAGGCGGTGACGGTGGGAGGTCTATATAAGCAGAGCTCGT
 TTAGTGAACCGGGCACTCAGATTCTGCGGTCTGAGTCCCTTCTCTGCTGGGCTGAAAAGG
 CCTTTGTAATAAATAAATTCTCTACTCAGTCCCTGTCTCTAGTTTGTCTGTTGAGATC
 CTACAGTTGGCGCCCGAACAGGGACCTGAGAGGGGCGCAGACCCTACCTGTTGAACCTGG
 CTGATCGTAGGATCCCGGGACAGCAGAGGAGAACTTACAGAAGTCTTCTGGAGGTGTTT
 30 CTGGCCAGAACACAGGAGGACAGGTAAGATTGGGAGACCCTTTGACATTGGAGCAAGGCG
 CTCAAGAAGTTAGAGAAGGTGACGGTACAAGGGTCTCAGAAATTAACCTACTGGTAACGT
 AATTGGGCGCTAAGTCTAGTAGACTTATTTATGATACCAACTTTGTAAAAGAAAAGGAC
 TGGCAGCTGAGGGATGTCATTCCATTGCTGGAAGATGTAACCTCAGACGCTGTCAGGACAA
 GAAAGAGAGGCCTTTGAAAGAACATGGTGGGCAATTTCTGTGTAAAGATGGGCCTCCAG
 35 ATTAATAATGTAGTAGATGGAAGGCATCATTCCAGCTCCTAAGAGCGAAATATGAAAAG
 AAGACTGCTAATAAAAAAGCAGTCTGAGCCCTCTGAAGAATATCTCTAGAAGTATGGATC
 CCCCAGGCTGCAGGAGTGGGAGGCACGATGGCCGCTTTGGTTCGAGGCGGATCCGGCCAT
 TAGCCATATTATTCATTGGTTATATAGCATAAATCAATATTGGCTATTGGCCATTGCATA
 CGTTGTATCCCATATGACATAATGTACATTTATATTGGCTCATGTCCAACATTACCGCCAT
 40 GTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTTATA
 GCCCATATATGGAGTTCGCGGTTACATAAATTACGGTAAATGGCCCGCTGGCTGACCGC
 CCAACGACCCCGCCCATGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAG
 GGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTAC
 ATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCG
 45 CCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACG
 TATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGAT
 AGCGGTTTGACTCAGGGGATTTCCAAGTCTCCACCCATTGACGTCAATGGGAGTTTGT
 TTTGGCACAAAATCAACGGGACTTTCAAAATGTCGTAACAACCTCCGCCCATTGACGC
 AAATGGGCGGTAGGCATGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTATGTAACC
 50 GTCAGATCGCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACC
 GATCCAGCCTCCGCGGCCCAAGCTTCAGCTGCTCGAGGATCTGCGGATCCGGGGAATTC
 CCCAGTCTCAGGATCCACCATGGGGGATCCCGTCGTTTTACACGTCGTGACTGGGAAAA
 CCCTGGCGTTACCCAATTATCGCCTTGACGACATCCCCCTTTCCGACGCTGGCGTAA
 TAGCGAAGAGGGCCCGACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATG
 55 GCGCTTTGCTGGTTTCCGGCACCAAGCGGTGCCGGAAGCTGGCTGGAGTGCATCT
 TCCTGAGGCGGATACTGTCGTCGTCCTTCAAACTGGCAGATGCACGGTTACGATGCGCC
 CATCTACACCAACGTAACCTATCCCAATTACGGTCAATCCGCGCTTTGTTCCACGGAGAA
 TCCGACGGGTTGTTACTCGCTCACATTTAATGTTGATGAAAGCTGGCTACAGGAAGGCCA
 GACGCGAATTATTTTGATGGCGTTAACTCGGCGTTTCATCTGTGGTGCAACGGGCGCTG
 60 GGTGCGTTACGGCCAGGACAGTCGTTTGGCGTCTGAATTTGACCTGAGCGCAATTTTACG
 CGCCGAGAAAAACCGCTCGCGGTGATGGTGCTGCGTTGGAGTGACGGCAGTTATCTGGA
 AGATCAGGATATGTGGCGGATGAGCGGCATTTCCGTGACGTCTCGTTGCTGCATAAACC
 GACTACACAAATCAGCGATTTCATGTTGCCACTCGCTTAATGATGATTCAGCCGCGC
 TGTACTGGAGGCTGAAGTTTACAGATGTGCGGCGAGTTGCGTGACTACCTACGGGTAACAGT
 65 TTCTTTATGGCAGGGTGAAACGCAAGGTGCGCCAGCGGACCGCGCCTTTCCGGCGGTGAAAT
 TATCGATGAGCGTGGTGGTTATGCCGATCGCGTCACACTACGTCTGAACGTCGAAAACCC
 GAAACTGTGGAGCGCCGAAATCCCGAATCTCTATCGTGCGGTGGTTGAACTGCACACCGC

ACGACGGACACGCTGATTGAAGCAGAAAGCCTGCGATGTCGGTTCGCGAGGTTGCGGATTGA
AAATGGTCTGCTGCTGCTGAACGGCAAGCCGTTGCTGATTCGAGGCGTTAACCGTCACGA
GCATCATCTCTGCATGGTCAGGTCATGGATGAGCAGACGATGGTGCAAGGATATCCTGCT
GATGAAGCAGAAACAATTTAACGCCGTGCGCTGTTGCGATTATCCGAACCATCCGCTGTG
GTACACGCTGTGCGACCGCTACGGCCTGTATGTGGTGGATGAAGCCAAATTATTGAACCCCA
CGGCATGGTGCCAAATGAATCGTGTGACCGATGATCCGCGCTGGCTACCGGCGATGAGCGA
ACGCGTAACGCGGAATGGTGACGCGCATCGTAATCACCCGAGTGTGATCATCTGGTCGCT
GGGGAATGAATCAGGCCACGGCGCTAATCACGACGCGTGATCGTGGATCAAACTCTGT
CGATCCTTCCCGCCCGGTGCAGTATGAAGGCGGCGGAGCCGACACCAGGCCACCGATAT
TATTTGCCCGATGTACGCGCGCGTGGATGAAGACAGCCCTTCCCGGCTGTGCCGAAATG
GTCCATCAAAAAATGGCTTTCGTACCTGGAGAGACGCGCCGCTGATCCTTTGCGAATA
CGCCACGCGATGGGTAAACAGTCTTGCGCTTTCGCTAAATATGGCAGGCGTTTCGTCA
GTATCCCGGTTTACAGGGCGGGCTTCGTCTGGGACTGGGTGGATCAGTCGCTGATTAAATA
TGATGAAACCGGCAACCGGTGGTCGGCTTACGGCGGTGATTTTGCGGATACGCCGAACGA
TCGCGAGTTCTGTATGAACGGTCTGTGCTTTGCCGACCGCAGCCGATCCAGCGCTGAGT
GGAAGCAAAACACGAGCAGCAGTTTTCCAGTTTCGTTTATCCGGGCAAAACCATCGAAGT
GACCAGCGAATACCTGTTCCGTCATAGCGATAACGAGCTCCTGCACTGGATGGTGGCGCT
GGATGGTAAGCCGCTGGCAACGCGTGAAGTGCCTCTGGATGTGCGCTCCCAAGGTAACAA
GTGGATTGAACCTGCTGAACACTACCGCAGCGGAGAGCGCGGCACTCTGGCTCACAGT
ACGCGTAGTGCAACCGAACGCGACCGCATGGTCAGAAAGCCGGGCATCAGCGCTGGCA
GCAGTGGCGCTCTGGCGGAAAACTCAGTGTGACGCTCCCGCGCGCTCCACGCCATCCC
GCATCTGACCCACCGCAAAATGGATTTTGCATCGAGCTGGGTAAATAAGCGTTGGCAATT
TAACCGCCAGTCAGGCTTCTTCTTACAGATGTGGATTGGCGATAAAAAACAACCTGTCAG
GCCGCTGCGCGATCAGTTTACCCGTGCACCGCTGGATAACGACATTGGCGTAAGTGAAGC
GACCCGATTGACCTTAACGCTTGGGTGCAACGCTGGAAGGCGCGGGCCATTACCAGGC
CGAAGCAGCGTTGTTGACGTGCACGCGAGATACACTTGTGATGCGGTGCTGATTACGAC
CGCTCACGCGTGGCAGCATCAGGGGAAAACTTATTTACGCCGAAAAACCTACCGGAT
TGATGGTAGTGGTCAAATGGCGATTACCGTTGATGTTGAAGTGGCGAGCGATACACCGCA
TCGGCGCGGATTGGCTGAACCTGCCAGCTGGCGAGGTAGCAGAGCGGGTAAACTGGTCT
CGGATTAGGGCCGCAAGAAAACTATCCGACGCGCTTACTGCCGCTGTTTGGACCGCTG
GGATCTGCCATTGTCAGACATGTATACCCCGTACGTCTTCCCGAGCGAAAAACGGTCTCG
CTGCGGGAGCGCGCAATTGAATTAATGGCCACACCAAGTGGCGCGGCGACTTCCAGTTCAA
CATCAGCCGCTACAGTCAACAGCAACTAGTGAAGAACAGCCATCGCCATCTGCTGCACCG
GGAAGAAGGCACATGGCTGAATATCGACGTTTTCATATGGGATTTGGTGGCGACGACTC
CTGGAGCCCGCTCAGTATCGGCGGAATTCAGCTGAGCGCCGGTCTGCTACCATTACCAGTT
GGTCTGGTGTCAAAAATAATAAACCAGGCGAGGGGGATCCGCAGATCCGGCTGTGGAA
GTGTGTCAGTTAGGGTGTGGAAGTCCCAAGCTCCCAAGCAGGCGAAGATGCAAAAG
CATGCCTGCAGGAATTCGATATCAAGCTTATCGATACCGTCGACCTCGAAGGGGGGGCCG
GTACCCAGCTTTTGTCCCTTATAGTAGGGTTAATTGCGCGGGAAGTATTTATCACTAAT
CAAGCACAAGTAAATCATGAGAACTTTTACTACAGCAAGCACAATCTCTCAAAAAATTT
GTTTTACAAAACTCCCTGGTGAACATGATTGGAAGGACCTACTAGGTGCTGTGGAAG
GGTGTATGGTGCAGTAGTAGTTAATGATGAAGGAAAGGGAATAATTGCTGTACCATTAACC
AGGACTAAGTTACTAATAAAAAACCAATTTAGTATTGTTGCAAGGAAGCAAGACCAACTAC
CATTTGTCAGCTGTTTCTGACCTCAATATTTGTTATAAGGTTTGATATGAATCCCAG
GGGAATCTCAACCCCTATTACCCAACAGTCAGAAAAATCTAAGTGTGAGGAGAACAATA
TTTTCAACCTTATTGTTATAATAATGACAGTAAGAACAGCATGGCAGAATCGAAGGAAGC
AGAGACCAAGAATGAACCTGAAGGAAGAACTCAAAAGAGAAAAAGAAAGATGACTGG
GGAAAAATAGGTATGTTTCTGTTATGCTTAGCAGGAAGTACTGGAAGGAATCTTTGGTGG
ATGAAGGACTCCACAGCAACATTATATAGGGTTGGTGGCGATAGGGGGAAGATTAAAC
GGATCTGGCCAAATCAAATGCTATAGAATGTGGGTTCCTTCCCGGGGTGTAGACCATTT
AAAAATGCTTCACTTATGATGACCAATAGAAGCATGCATATGGATAATAATACTGCTACA
TATTAGAAGCTTTAAACCAATATAACTGCTCTATAAATAACAAAAAGAAATAGAAACAT
GGAAGTTAGTAAAGACTTCTGGCATAACTCCTTTACCTATTTCTTCTGAAGCTAACACTG
TACTAATTAGACATAAGAGAGATTTTGGTATAAGTGCAATAGTGGCAGCTATTGTAGCCG
TACTGCTATTGCTGCTAGCGCTACTATGTCTTATGTTGCTCTAACTGAGGTTAACAAAA
AATGGAAGTACAAAAATCACTTTTGAAGGTAGAAAAATAGTACTCTAAATGGTATGGATT
AATAGAACGACAAATAAAGATATTATATGCTATGATTTCTCAAACACATGCAGATGTTT
ACTGTTAAGGAAGACCAACAGGTAGAGGAGACATTTAATTAATTTGGATGTTATAGAAA
AACCATGATGATTTTGTCTACTGGTCACTCCCTGGAATATGTCATGGGACATTTAAATG
GTCAACACAATGGGATGACTGGGTAAAGCAAAATGGAAGATTTAAATCAAGAGATACTAA
TACACTTTCATGGAGCCAGGAACAATTTGGCACAATCCATGATAACATTCAATACACCAG
TAGTATAGCTCAATTTGGAAGAACCTTTGGAGTCATATTGGAATTTGGATTTCTGGAT
GGGAGCTTCCATTATAAAATATATAGTATGTTTTGCTTATTTATTTGTTACTAACT
TTGCGCTAAGATCCTCAGGGCCCTCTGGAAGGTGACCAGTGGTGCAGGCTCCTCCGGCA
TCGTTTACCTGAAGAAAAAATTCATCAACAAACATGCATCGCGAGAAGACACCTGGGAG
GGCCCAACACAAACATACACCTAGCAGGCGTACCGGTGATCAGGGGACAAATACTACA
GCAGAAGTACTCCAGGAACGACTGGAATGGAGAATCAGAGGAGTACAACAGGCGGCCAA
GAGCTGGGTGAAGTCAATCGAGGCAATTTGGAGAGAGCTATATTTCCGAGAAGACCAAAG
GGAGATTTCTCAGCTTGGGGGGCTATCAACGAGCACAAGAACGCGCTTGGGGGGAACA
TCCTCAACAAGGTCTTAGACCTGGAGATTGCAAGCGAAGGAGGAACAATTATGACT
TTGCATTAAAGCCCAAGAAGGAACTCTCGCTATCCCTTGTGTGGATTTCCCTTATGGC
ATTTTGGGGACTAGTAATATAGTAGGACGCATAGCAGGCTATGGATTACGTGGAGACTCG
GTTTATAATAAGGATTTGATTAGAGGCTTAAATTTGATATTTGAAATTAATCAGAAAAA
GCTTGATTATATTGGAAGAGCTTTAAATCCTGGACATCTCATGTATCAATGCTCAGT

ATGTTTAGAAAAACAAGGGGGGAAGTGTGGGGTTTTATGAGGGGTTTTATAAATGATTA
 TAAGAGTAAAAAGAAAGTTGCTGATGCTCTCATAACCTTGTATAACCCAAAGGACTAGCT
 CATGTTGCTAGGCAACTAAACCGCAATAACCGCATTGTGTACGCGAGTTCCCATTTGGTG
 ACGCGTTAACTTCTGTTTTTACAGTATATAAGTGCTTGTATTCTGACAATTGGGCACTC
 5 AGATTCTGCGGTCTGAGTCCCTTCTCTGCTGGGCTGAAAAGGCCTTTGTAATAAATATAA
 TTCTCTACTCAGTCCCTGTCTCTAGTTTGTCTGTTGAGATCCTACAGAGCTCATGCCTT
 GGCCTAATCATGGTCAATAGCTGTTTCTGTGTGAAATTGTTATCCGCTCACAAATCCACA
 CAACATACGAGCCGGAAGCATAAAGTGTAAGCCCTGGGGTGCCTAATGAGTGAGCTAACT
 10 CACATTAATTGCGTTGCGCTCACTGCCCCGCTTCCAGTCGGGAAACCTGTCGTGCCAGCT
 GCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCCTATTGGGCGCTCTTCCGC
 TTCCTCGCTCACTGACTCGCTGCGCTCGGTCTGCTCGGCTGCGGCGAGCGGTATCAGTCA
 CTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAATCATGTG
 AGCAAAAGGCCAGCAAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCA
 TAGGCTCCGCCCCCTGACGAGCATCAAAAAATCGACGCTCAAGTCAGAGGTGGCGAAA
 15 CCGGACAGGACTATAAAGATACAGGCGTTTCCCCCTGGAAGCTCCCTCGTGGCGCTCTCC
 TGTTCGACCCCTGCCGTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGCC
 GCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTGCTTCCGCTCCAAGCT
 GGGCTGTGTGCAGCAACCCCGTTACGCCCCGACCGCTGCGCCTTATCCGTTAACTATCG
 TCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAG
 20 GATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTA
 CGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGG
 AAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAACAAACCCGCTGGTAGCGGTGGTTTTTT
 TGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTT
 TTCTACGGGGTCTGACGCTCAGTGGAACGAAAACCTACGTTAAGGGATTTTGGTCATGAG
 25 ATTATCAAAAAGGATCTTACCTAGATCCTTTAAATTAATAAATGAAGTTTAAATCAAT
 CTAAAGTATATATGAGTAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACC
 TATCTCAGCGATCTGTCTATTTCTGTTTATCCATAGTTGCTGACTCCCCGTCGTGTAGAT
 AACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCC
 ACGCTCACCGGCTCCAGATTTATCAGCAATAAACCCAGCCAGCCGGAAGGGCCGAGCGCAG
 30 AAGTGGTCTGCAACTTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAG
 AGTAAGTAGTTCGCCAGTTAATAGTTTGCAGCAACGTTGTTGCCATTGCTACAGGCATCGT
 GGTGTCACGCTCGTCGTTTGGTATGGCTTCATTAGCTCCGGTTCCTAACGATCAAGGCG
 AGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGCTCCTCCGATCGT
 35 TGTCAGAAGTAAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTC
 TCTTACTGTATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTC
 ATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGGCCGGCGTCAATACGGGATAA
 TACCGCGCCACATAGCAGAACTTTAAAGTGCTCATCATTGGAACCGTTCTTGGGGGCG
 AAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTTCGATGTAACCCACTCGTGCACC
 CAACTGATCTTACGATCTTTTACTTTTACCAGCGTTTCTGGGTGAGCAAAAAACAGGAAG
 40 GCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAATGTTGAATACTCATACTCTT
 CCTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATT
 TGAATGTATTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCC
 ACCTAAATTGTAAGCGTTAATATTTTGTAAAAATTCGCGTTAAATTTTTGTAAATCAGC
 TCATTTTATAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAAGAAATAGACC
 45 GAGATAGGGTTGAGTGTGTTCCAGTTTGAACAAGAGTCCACTATTAAGAACGTGGAC
 TCCAACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCATCA
 CCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCTAAAGGG
 AGCCCCGATTTAGAGCTTGACGGGGAAGCCAACCTGGCTTATCGAAATTAATACGACT
 CACTATAGGGAGACCGGC

SEQ ID NO 2

pONY8.0G

5 AGATCTTGAATAATAAAATGTGTGTTTGTCCGAAATACGCGTTTTGAGATTTCTGTGCGC
 GACTAAATTCATGTGCGCGGATAGTGGTGTATCGCCGATAGAGATGGCGATATTGGAA
 AAATTGATATTTGAAAATATGGCATATTGAAAATGTGCGCGATGTGAGTTTCTGTGTAAC
 TGATATCGCCATTTTCCAAAAGTGATTTTGGGCATACGCGATATCTGGCGATAGCGCT
 10 TATATCGTTTACGGGGGATGGCGATAGACGACTTTGGTGACTTGGGCGATTCTGTGTGTC
 GCAAATATCGCAGTTTCGATATAGGTGACAGACGATATGAGGCTATATCGCCGATAGAGG
 CGACATCAAGCTGGCACATGGCCAATGCATATCGATCTATACATTGAATCAATATTGGCC
 ATTAGCCATATTATTCATTGGTTATATAGCATAAATCAATATTGGCTATTGGCCATTGCA
 TACGTTGTATCCATATCGTAATATGTACATTTATATTGGCTCATGTCCAACATTACCGCC
 15 ATGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCA
 TAGCCCATATATGGAGTTCGCGTTACATAAATTACGGTAAATGGCCCGCTGGCTGACC
 GCCCAACGACCCCGCCCATTTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAAT
 AGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGT
 ACATCAAGTGTATCATATGCCAAGTCCGCCCCCTATTGACGTCAATGACGGTAAATGGCC
 CGCCTGGCATTATGCCAGTACATGACCTTACGGGACTTTCTACTTGGCAGTACATCTA
 20 CGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACACCAATGGGCGTGG
 ATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCATTGACGTCAATGGGAGTTT
 GTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCTGTAACAACTGCGATCGCCCGCC
 CGTTGACGCAAAATGGGCGGTAGGCGGTGACGGTGGGAGGTCTATATAAGCAGAGCTCGT
 TTAGTGAACCGGGCACTCAGATTCTGCGGTCTGAGTCCCTTCTCTGCTGGGCTGAAAAGG
 25 CCTTTGTAATAAATATAATTCTCTACTCAGTCCCTGTCTCTAGTTTGTCTGTTGAGATC
 CTACAGTTGGCGCCCGAACAGGGACCTGAGAGGGGCGCAGACCCTACCTGTTGAACCTGG
 CTGATCGTAGGATCCCCGGGACAGCAGAGGAGAAGTTACAGAAGTCTTCTGGAGGTGTTCT
 CTGGCCAGAACACAGGAGGACAGGTAAGATTGGGAGACCCTTTGACATTGGAGCAAGGGC
 CTCAAGAAGTTAGAGAAGGTGACGGTACAAGGGTCTCAGAAATTAACACTGGTAACTGT
 30 AATTGGGCGCTAAGTCTAGTAGACTTATTTTCATGATACCAACTTTGTAAGAAAAGGAC
 TGGCAGCTGAGGGATGTCATTCCATTGCTGGAAGATGTAACCTCAGACGCTGTCAGGACAA
 GAAAGAGAGGCCTTTGAAAGAACATGGTGGGCAATTTCTGCTGTAAAGATGGGCCTCCAG
 ATTAATAATGTAGTAGATGGAAGGCATCATTCAGCTCCTAAGAGCGAAATATGAAAAG
 AAGACTGCTAATAAAAAAGCAGTCTGAGCCCTCTGAAGAATATCTCTAGAACTAGTGGATC
 35 CCCCCGGGTGCAAGGAGTGGGGAGGCACGATGGCCGCTTTGGTCGAGGCGGATCCGGCCAT
 TAGCCATATTATTCATTGGTTATATAGCATAAATCAATATTGGCTATTGGCCATTGCATA
 CGTTGTATCCATATCATAATATGTACATTTATATTGGCTCATGTCCAACATTACCGCCAT
 GTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATA
 40 GCCCATATATGGAGTTCGCGGTTACATAAATTACGGTAAATGGCCCGCTGGCTGACCGC
 CCAACGACCCCGCCCATTTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAG
 GGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTAC
 ATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCG
 CCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACG
 45 TATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGAT
 AGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCATTGACGTCAATGGGAGTTTGT
 TTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCTGTAACAACTCCGCCCATTTGACGC
 AAATGGGCGGTAGGCGATGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTAGTGAACC
 GTCAGATCGCCTGGAGACGCCATCCACGCTGTTTGGACCTCCATAGAAGACACCGGGACC
 50 GATCCAGCCTCCGCGCCCAAGCTTGTGGGATCCACCGGTGCGCCACCATGGTGAGCAA
 GGGCGAGGAGCTGTTACCGGGGTGGTGGCCATCCTGGTCGAGCTGGACGGCGACGTAAA
 CGGCCACAAGTTACGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGAC
 CCTGAAGTTCATCTGCACACCGGCAAGCTGCCCGTGCCCTGGCCACCCCTCGTGACCAC
 55 CCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCATGAAGCAGCAGCACTT
 CTTCAGTCCGCCATGCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGA
 CGGCAACTACAAGACCCGCGCGGAGGTGAAGTTCGAGGGGCGACACCCTGGTGAACCGCAT
 CGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGACAAGCTGGAGTA
 CAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGT
 60 GAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCA
 GCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCGACAACCACTACCTGAGCAC
 CCAGTCCGCCCTGAGCAAAAGACCCCAACGAGAAGCGCGATCACATGGTCTGGAGTT
 CGTGACCGCGCGGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAGCGGGCCGCA
 CTCTAGAGTGCACCTGCAGGCATGCAAGCTTCAGCTGCTCGAGGGGGGGGCCCGGTACCCA
 65 GCTTTTGTCCCTTTAGTGAGGGTTAATTGCGCGGAAGTATTTATCACTAATCAAGCAC
 AAGTAATACATGAGAACTTTTACTACGAACAAGCAACATCCTCCAAAAAATTTGTTTTT
 ACAAATCCCTGGTGAACATGATTGGAAGGGACCTACTAGGGTGTGTGGAAGGGTGATG
 GTGCAGTAGTAGTTAATGATGAAGGAAAGGGAATAATTGCTGTACCATTAACCAGGACTA
 AGTTACTAATAAAAAAATTTGAGTATTGTTGCAAGGAAGCAAGCAACCACTACCATTTGTC
 70 AGCTGTGTTTCTGACCTCAATATTTGTTATAAGGTTTGATATGAATCCAGGGGGGAATC
 TCAACCCCTATTACCCAACAGTCAGAAAAATCTAAGTGTGAGGAGAACAATGTTTCAA
 CCTTATTGTTATAATAATGACAGTAAGAACAGCATGGCAGAATCGAAGGAAGCAAGAGAC

CAAGAATGAACCTGAAAGAAGAAATCTAAAGAAGAAAAAGAAGAAATGACTGGTGGAAAA
 TAGGTATGTTTCTGTTATGCTTAGCAGGAACACTGAGGAATACTTTGGTGGTATGAAG
 GACTCCCAAGCAACATTATATAGGGTTGGTGGCGATAGGGGGAAGATTAAACGGATCTG
 5 GCCAATCAAATGCTATAGAATGCTGGGGTTCCTTCCCGGGGTGTAGACCATTTCAAAAATT
 ACTTCAGTTATGAGACCAATAGAAGCATGCATATGGATAATAAATACTGCTACATTATTAG
 AAGCTTTAACCAATAAAGTCTCTATAAATAACAAAACAGAATTAGAAACATGGAAGTT
 AGTAAAGACTTCTGGCATAACTCCTTTACCTATTCTTCTGAAGCTAACACTGGACTAAT
 TAGACATAAGAGAGATTTTGGTATAAGTGAATAGTGGCAGCTATTGTAGCCGCTACTGC
 TATTGCTGCTAGCGCTACTATGTCTTATGTTGCTCTAACTGAGGTAAACAAAATAATGGA
 10 AGTACAAAATCATACTTTTGGAGTAGAAAAATAGTACTCTAAATGGTATGGATTTAATAGA
 ACGACAAAATAAAGATATTATATGCTATGATTCTTCAAACACATGCAGATGTTCAACTGTT
 AAAGGAAAGACAACAGGTAGAGGAGACATTTAATTTAATTGGATGTATAGAAAAGAACACA
 TGTATTTTGTCACTAGGTCATCCCTGGAATATGTCATGGGGACATTTAAATGAGTCAAC
 ACAATGGGATGACTGGGTAAGCAAAATGGAAGATTAAATCAAGAGATACTAACTACACT
 15 TCATGGAGCCAGGAACAATTTGGCACAATCCATGATAACATTCAATACACCAGATAGTAT
 AGCTCAATTTGGAAAAGACCTTTGGAGTCATATTGGAATTTGGATTCTGGATTGGGAGC
 TTCCATTATAAAATATATAGTGATGTTTTTGTCTATTTATTTGTTACTAACCTCTTCGCC
 TAAGATCTCAGGGCCCTCTGGAAGGTGACCAGTGGTGCAGGGTCTCCGGCAGTCGTTA
 CCTGAAGAAAAAATTCATCACAAACATGCATCGCGAGAAGACACCTGGGACCAGGCCCA
 20 ACACAACATACACCTAGCAGGCGTGACCGGTGGATCAGGGGACAAATACTACAAGCAGAA
 GTACTCCAGGAACGACTGGAATGGAGAATCAGAGGAGTACAACAGGCGGCCAAAGAGCTG
 GGTGAAGTCAATCGAGGCAATTTGGAGAGAGCTATATTTCCGAGAAGACCAAGGGGAGAT
 TTCTCAGCCTGGGGCGGCTATCAACGAGCACAAGAACGGCTCTGGGGGAACAATCCTCA
 CCAAGGGTCTTAGACCTGGAGATTGGAAGCAAGGAGGAAACATTTATGACTGTTGCAT
 25 TAAAGCCCAAGGAAGCAACTCTCGCTATCCCTTGTGTGGATTTCCTTATGGCTATTTTG
 GGGACTAGTAATTATAGTAGGACGCATAGCAGGCTATGGATTACGTGGACTCGCTGTTAT
 AATAAGGATTTGTATTAGAGGCTTAAATTTGATATTTGAAATAATCAGAAAAATGCTTGA
 TTATATTGGAAGAGCTTTAAATCCTGGCACATCTCATGTATCAATGCCTCAGTATGTTTA
 GAAAAACAAGGGGGAACCTGTGGGGTTTTATGAGGGTTTTATAAATGATTATAAGAGT
 30 AAAAAAGAAAGTTGCTGATGCTCTCATAACCTTGTATAACCCAAAGGACTAGCTCATGTTG
 CTAGGCAACTAAACCGCAATAACCGCATTTGTGACGCGAGTTCCCCATTGGTGACGCGTT
 AACTTCTGTGTTTTACAGTATATAAGTGCTTGTATTCTGACAATTGGGCACTCAGATTCT
 GCGGTCTGAGTCCCTTCTCTGTGGCTGAAAAGGCCTTTGTAATAAATAAATTTCTCTA
 CTCAGTCCCTGTCTCTAGTTTGTCTGTTTCGAGATCCTACAGAGCTCATGCCTTGGCGTAA
 35 TCATGGTCAATAGCTGTTTCTGTGTGAAATTGTTATCCGCTCACAATTCACACAACATA
 CGAGCCGGAAGGAGCAAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGTAACACATTA
 ATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAA
 TGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCCTATTGGGCGCTCTTCCGCTTCCCTCG
 CTCAGTACTCGCTGCGCTCGGTCTGCTGCGGCGAGCGGTATCAGCTCACTCAAAAG
 40 CCGGTAATACGGTTATCCACAGAAATCAGGGGATAACGCAAGGAAAGAACATGTGAGCAAAA
 GGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTC
 CGCCCCCTGACGAGCATCAAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACA
 GGACTATAAAGATACCGCGTTTCCCTTGGAGCTCCCTCGTGCCTCTCCTGTTCCG
 ACCCTGCCGCTTACCGGATACCTGTCCGCTTTTCTCCCTCGGGAAGCGTGCGCTTTTCT
 45 CATAGCTCACGCTGTAGGTATCTCAGTTTCGGTGTAGGTCTGCTCCAAGCTGGGCTGT
 GTGCACGAACCCCCCGTTACGCCGACCGCTGCGCTTATCCGGTAACATATCGTCTTGAG
 TCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGC
 AGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTAC
 ACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAAGA
 50 GTTGGTAGCTCTTGTCCGGCAAAACAAACACCGCTGGTAGCGGTGGTTTTTTTGTGTTGC
 AAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACG
 GGGTCTGACGCTCAGTGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCA
 AAAAGGATCTTACCTAGATCCTTTTAAATTAATAAATGAAAGTTTAAATCAATCTAAAGT
 ATATATGAGTAAAGCTTTGCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCA
 55 GCGATCTGTCTATTTCTGTTTATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAAAGTACG
 ATACGGGAGGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCA
 CCGGCTCCAGATTTATCAGCAATAAACAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGT
 CCTGCAACTTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGT
 AGTTCGCCAGTTAATAGTTTGCAGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCA
 60 CGCTCGTCTGTTGGTATGGCTTCACTAGCTCCGGTTCCCAACGATCAAGGCGAGTTACA
 TGATCCCCCATGTTGTGCAAAAAAGCGTTAGCTCCTTCGGTCTCCGATCGTTGTGAGA
 AGTAAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACT
 GTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAAGTACTCAACCAAGTCATTCTGA
 GAATAGTGTATGCGGCGACCGAGTTGCTCTTGGCCGGCGTCAATACGGGATAATACCGCG
 65 CCATAGCAGAACTTTAAAGTGCTCATCTATTGGAACGTTCTTCGGGGCAAACTC
 TCAAGGATCTTACCGCTGTTGAGATCCAGTTTCGATGTAACCCACTCGTGACCCCACTGA
 TCTTCAGCATCTTTACTTTTACCAGCGTTTCTGGGTGAGCAAAAAACAGGAAGGCAAAAT
 GCCGCAAAAAAGGGAATAAGGGCGACACGGAATGTTGAATACTCATACTCTTCTTTTTT
 CAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGT
 70 ATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCGGAAAAAGTGCCACCTAAA
 TTGTAAGCGTTAATATTTTGTAAAAATTCGCGTTAAATTTTGTAAATCAGCTCATTTT
 TTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAAAGATAGACCGAGATAG
 GGTGAGTGTGTTCCAGTTTGGAAACAGAGTCCACTATTAAGAACGTGGACTCCAACG
 TCAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCACTACGTGAACCATCACCTAAT

CAAGTTTTTGGGGTCGAGGTGCCGTAAGCACTAAATCGGAACCCTAAAGGGAGCCCC
GATTTAGAGCTTGACGGGAAAGCCAACCTGGCTTATCGAAATTAATACGACTCACTATA
GGGAGACCGGC

5

SEQ ID NO 3 pONY 8.0Z 5' pos del CTS

10 AGATCTTGAATAATAAAATGTGTGTTTGTCCGAAATACGCGTTTTGAGATTTCTGTGCGC
GACTAAATTCATGTGCGCGCATAGTGGTGTATTCGCGGATAGAGATGGCGATATTGGAA
AAATTGATATTTGAAAATATGGCATATTGAAAATGTCGCGCATGTGAGTTTCTGTGTAAC
TGATATCGCCATTTTCCAAAAGTGATTTTGGGCATACGCGATATCTGGCGATAGCGCT
TATATCGTTTACGGGGGATGGCGATAGACGACTTTGGTGACTTTGGCGGATTCTGTGTGTC
15 GCAAAATATCGCAGTTTCGATATAGGTGACAGACGATATGAGGCTATATCGCCGATAGAGG
CGACATCAAGCTGGCACATGGCCAATGCATATCGATCTATACATTGAATCAATATTGGCC
ATTAGCCATATTATTCATTGGTTATATAGCATAAATCAATATTGGCTATTGGCCATTGCA
TACGTTGTATCCATATCGTAATATGTACATTTATATTGGCTCATGTCCAACATTACCGCC
ATGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCA
20 TAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACC
GCCCACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAAT
AGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGT
ACATCAAGTGTATCATATGCCAAGTCCGCCCTTATTGACGTCAATGACGGTAAATGGCC
CGCCTGGCATTATGCCAGTACATGACCTTACGGGACTTTCCCTACTTGGCAGTACATCTA
25 CGTATTAGTCAATAAAGTACCATGGTGATGCGGTTTTGGCAGTACACCAATGGCGCTGG
ATAGCGGTTTTGACTCACGGGGATTTCGAAGTCTCCACCCATTGACGTCAATGGGAGTTT
GTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACCTGCGATCGCCCGCC
CCGTTGACGCAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGT
TTAGTGAACCGGGCACTCAGATTCTGCGGTCTGAGTCCCTTCTCTGCTGGGCTGAAAAGG
30 CCTTTGTAATAAATATAATTCTCTACTCAGTCCCTGTCTCTAGTTTGTCTGTTCGAGATC
CTACAGTTGGCGCCCGAACAGGGACCTGAGAGGGGCGCAGACCCCTACCTGTTGAACCTGG
CTGATCGTAGGATCCCCGGGACAGCAGAGGAGAACCTACAGAAGTCTCTGGAGGTGTTCT
CTGGCCAGAACACAGGAGGACAGGTAAAGATTGGGAGACCCTTTGACATTGGAGCAAGGCG
CTCAAGAAGTTAGAGAAGGTGACGGTACAAGGGTCTCAGAAATTAACCTACTGGTAACTGT
35 AATTGGCGCTAAGTCTAGTAGACTTATTTATGATACCAACTTTGTAAAAGAAAAGGAC
TGGCAGCTGAGGGATGTCAATCCATTGCTGGAAGATGTAACCTCAGACGCTGTGAGGACAA
GAAAGAGAGGCCCTTTGAAAGAACATGGTGGCAATTCTGCTGTAAAGATGGGCCTCCAG
ATTAATAATGTAGTAGATGGAAGGCATCATTCAGCTCCTAAGAGCGAAATATGAAAAG
AAGACTGCTAATAAAAAGCAGTCTGAGCCCTCTGAAGAATATCTCTAGAGTCGACGCTCT
CATTACTTGTAAACAAAGGAGGAAAGTATGGGAGGACAGACACCATGGGAAGTATTTAT
40 CACTAATCAAGCACAAGTAATACATGAGAACTTTTACTACAGCAAGCACAATCCTCCAA
AAAATTTGTTTTTACAAAATCCCTGGTGAACATGGTCTGACTCTAGAAGTGTGGATCCC
CCGGCTCGAGGATGGGGAGGCACGATGGCCGCTTTGGTCGAGGCGGATCCGGCCATTA
GCCATATTATTATTGTTTATATAGCATAAATCAATATTGGCTATTGGCCATTGCATACG
45 TTGTATCCATATCATAATATGTACATTTATATTGGCTCATGTCCAACATTACCGCCATGT
TGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTTATAGC
CCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCC
AACGACCCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGG
ACTTTCCATTGACGTCAATGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACAT
50 CAAGTGATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCC
TGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTA
TTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAG
CGGTTTGAATCAGGGGATTTCCAAGTCTCCACCCATTGACGTCAATGGGAGTTTGT
TGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACCTCCGCCCATTGACGCAA
55 ATGGGCGGTAGGCATGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTGTGAAACCGT
CAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACGGGACCGA
TCCAGCCTCCGCGGCCCCAAGCTTCAGCTGCTCGAGGATCTGCGGATCCGGGGAATTCCC
CAGTCTCAGGATCCACCATGGGGATCCCGTCGTTTTACAACGTCGTGACTGGGAAAACC
CTGGCGTTACCCAACCTAATCGCCTTGACGACATCCCCCTTTGCCAGCTGGCGTAATA
60 GCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGC
GCTTTGCGCTGGTTTCCGGCACAGAACGGTGCCGGAAAGCTGGCTGGAGTGCGATCTTC
CTGAGGCCGATACGTGCTGCTCCCCCAAACCTGGCAGATGCACGGTTACGATGCGCCCA
TCTACACCAACGTAAACCTATCCATTACGGTCAATCCGCCGTTTTGTTCCACGGAGAATC
CGACGGGTTGTTACTCGCTCACATTTAATGTTGATGAAAGCTGGCTACAGGAAGGCCAGA
CGCGAATTATTTTTGATGGCGTTAACTCGGCGTTTCATCTGTGGTGCAACGGGCGCTGGG
65 TCGGTTACGGCCAGGACAGTCGTTTCCCGTCTGAATTTGACCTGAGCGCATTTTTACGCG
CCGGAGAAACCGCCTCGCGGTGATGGTGCTGCGTTGGAGTGACGGCAGTTATCTGGAAG
ATCAGGATATGTGGCGGATGAGCGGCATTTCCCGTGACGTCTCGTTGCTGCATAAACCGA
CTACACAAATCAGCGATTTCCATGTTGCCACTCGCTTTAATGATGATTTAGCCCGCGCTG
TACTGGAGGCTGAAGTTAGATGTGCGGCGAGTTGCGTGACTACCTACGGGTAAACAGTTT
70 CTTTATGGGCTGAACAGCAGGTCGCCAGCGGCACCGCCCTTTCCGCGGTGAATTA
TCGATGAGCGTGGTGGTTATGCCGATCGCGTCACACTACGCTCTGAACGTCGAAAACCGA

AACTGTGGAGCGCCGAAATCCCGAATCTCTATCGTGC GGTTGAACTGCACACCGCCG
 ACGGCACGCTGATTGAAGCAGAAGCCTGCGATGTCGGTTTCGCGAGGTGCGGATTGAAA
 ATGGTCTGCTGCTGCTGAACGGCAAGCCGTTGCTGATTTCGAGGCGTTAACCGTCACGAGC
 5 ATCATCCTCTGCATGGTCAGGTCATGGATGAGCAGACGATGGTGCAGGATATCCTGCTGA
 TGAAGCAGAACAACTTTAACGCCGTGCGCTGTTCGCATTATCCGAACCATCCGCTGTGGT
 ACACGCTGTGCGACCGCTACGGCCTGTATGTGGTGGATGAAGCCAATATTGAAACCCACG
 GCATGGTGCCAATGAATCGTCTGACCGATGATCCGCGCTGGCTACCGCGCATGAGCGAAC
 GCGTAACGCGAATGGTGCAGCGCATCGTAATCACCCGAGTGTGATCATCTGGTCGCTGG
 10 GGAATGAATCAGGCCACGGCGCTAATCACGACGCGCTGTATCGCTGGATCAAATCTGTCTG
 ATCCTTCCCGCCCGGTGCAGTATGAAGCGGGGAGCCGACACACGCGCCACCGATATTA
 TTTGCCCGATGTACGCGCGCTGGATGAAGACCAGCCCTTCCCGCTGTGCCGAAATGGT
 CCATCAAAAAATGGCTTTCGCTACCTGGAGAGACGCGCCCGCTGATCCTTTGCGAATACG
 CCCACGCGATGGGTAACAGTCTTGGCGGTTTCGCTAAATACTGGCAGGCGTTTCGTCAGT
 15 ATCCCGTTTACAGGGCGGCTTCGTCTGGGACTGGGTGGATCAGTCGCTGATTAAATATG
 ATGAAACGGCAACCCGTGGTTCGGCTTACGGCGGTGATTTTGGCGATACGCCGAACGATC
 GCCAGTTCTGTATGAACGGTCTGGTCTTTCGCGACCGCACGCCGCATCCAGCGCTGACGG
 AAGCAAAACACCAAGCAGCAGTTTTTTCCAGTTCCGTTTATCCGGGCAACCATCGAAGTGA
 CCAGCGAATACCTGTTCCGTCATAGCGATAACGAGCTCCTGCACTGGATGGTGGCGCTGG
 20 ATGGTAAGCCCGTGGCAAGCGGTGAAGTGCTCTGGATGTGCTCCACAAGGTAAACAGT
 TGATTGAAGTGCCTGAATACCGCAGCCGAGAGCGCCGGGCAACTCTGGCTCAGAGTAC
 CGGTAGTGCAACCGAAGCGGACCGCATGGTCAGAAGCCGGGCACATCAGCGCCTGGCAGC
 AGTGGCGTCTGGCGGAAAACCTCAGTGTGACGCTCCCGCCGCGTCCCACGCCATCCCGC
 ATCTGACCACCGCGAAATGGATTTTGCATCGAGCTGGGTAATAAGCGTTGGCAATTTA
 25 ACCGCCAGTCAGGCTTTTTCACAGATGTGGATTGGCGATAAAAAACAAGTCTGACGC
 CGCTGCGCGATCAGTTTACCCGTGACCGCTGGATAACGACATTGGCGTAAGTGAAGCGA
 CCCGCAATTGACCTAACGCGCTGGGTGCAACGCTGGAAGGCGCGGGCCATTACAGGCCG
 AAGCAGCGTGTGTGAGTGCACGGCAGATACACTTGTGATGCGGTGCTGATTACGACCG
 CTCACGCGTGGCAGCATCAGGGGAAAACCTTATTTATCAGCCGAAAACCTACCGGATTG
 30 ATGGTAGTGGTCAAATGGCGATTACCGTTGATGTTGAAGTGGCGAGCGATACACCGCATC
 CGGCGCGGATTGGCCTGAAGTGCAGCTGGCGCAGGTAGCAGAGCGGGTAAACTGGCTCG
 GATTAGGCGCGCAAGAAAACCTATCCCGACCGCCTTACTGCCGCTGTTTTGACCGCTGGG
 ATCTGCCATTGTGACAGATGTATACCCCGTACGCTCTCCCGAGCGAAAACGCTGCGCT
 GCGGGACGCGGAATTGAATTATGGCCACACAGTGGCGCGGCGACTTCCAGTTCAACA
 35 TCAGCCGCTACAGTCAACAGCAACTGATGGAAACCAGCCATCGCCATCTGCTGCACGCGG
 AAGAAGGCACATGGCTGAATATCGACGGTTTCCATATGGGGATTGGTGGCGACGACTCCT
 GGAGCCCGTCAGTATCGGCGGAATTCCAGCTGAGCGCCGGTCTGCTACCATTAACAGTTGG
 TCTGCTGTCAAAAATAATAAACCAGGCGAGGGGGGATCCGCAGATCCGGCTGTGGAATG
 40 TGTGTCAGTTAGGGTGTGGAAGTCCCGAGCTCCCGCAGGCGAGAAGTATGCAAGCA
 TGCCTGCAGGAATTCGATATCAAGCTTATCGATACCGTGCACCTCAGGGGGGGCCCGGA
 CCTACTAGGGTGTGTTGAAGGGTGTGTTGTCAGTAGTAGTTAATGATGAAGGAAAGGGA
 ATAATTGCTGTACCATTAACAGGACTAAGTTACTAATAAAACCAATAGTATTGTTG
 CAGGAAGCAAGACCCCACTACCATTTGTCAGCTGTGTTTCTGACCTCAATATTTGTTATA
 45 AGGTTTGATATGAATCCAGGGGAATCTCAACCCCTATTACCCACAGTCAGAAAAATC
 TAAGTGTGAGGAGAACACAATGTTTCAACCTTATGTTATAATAATGACAGTAAGAACAG
 CATGGCAGAAATCGAAGGAAGCAAGAGACCAAGAAATGAACCTGAAAGAAGAACTAAAGAA
 GAAAAAGAAGAAATGACTGGTGGAAAATAGGTATGTTTCTGTTATGCTTAGCAGGAAT
 ACTGGAGGAATACCTTGGTGGTATGAAGGACTCCACAGCAACATATATAGGGTGGTG
 50 CGGATAGGGGGAAGATTAAACGGATCTGGCCAATCAAATGCTATAGAATGCTGGGGTTCC
 TTCCCGGGGTGTAGACCATTTCAAAATTACTTCAGTTATGAGACCAATAGAAGCATGCAT
 ATGGATAATAATACTGCTACATTATTAGAAGCTTTAACCAATATAACTGCTCTATAAATA
 ACAAACAGAAATTAGAAACATGGAAGTTAGTAAAGACTTCTGGCATAACTCCTTTACCTA
 TTTCTTCTGAAGCTAACACTGGACTAATTAGACATAAGAGAGATTTTGGTATAAGTGCAA
 TAGTGGCAGCTATTGTAGCCGCTACTGCTATTGCTGCTAGCGCTACTATGCTTATGTTG
 55 CTCTAACTGAGGTTAACAAAATAATGGAAGTACAAAATCATACTTTTGGGTAGAAAATA
 GTACTCTAAATGGTATGGATTAAATAGAACGACAAATAAGATATTATATGCTATGATTC
 TTCAAACACATGCAGATGTTCAACTGTTAAAGGAAAGACAACAGGTAGAGGAGACATTTA
 ATTTAATTGGATGTATAGAAAGAACACATGTATTTTGTCACTGGTTCATCCCTGGAATA
 TGTGATGGGGACATTTAAATGAGTCAACACATGGGATGACTGGGTAAGCAAAATGGAAG
 60 ATTTAAATCAAGAGATACTAATACTACACTTCATGGAGCCAGGAACAATTTGGCACAATCCA
 TGATAACATTCAATACACCAGATAGTATAGCTCAATTTGGAAAAGACCTTTGGAGTCATA
 TTGGAATTTGGATTCTCGGATTGGGAGCTTCCATTATAAAATATATAGTGATGTTTTGCT
 TTATTTATTTGTTACTAACCTCTTCGCTAAGATCCTCAGGGCCCTCTGGAAGGTGACCA
 65 GTGGTGCAGGGTCTCCGGCAGTCGTTACCTGAAGAAAAAATTCATCACAAACATGCAT
 CGCGAGAAGACACCTGGGACCAGGCCAACACAACATACCTAGCAGGCGTGACCGGTG
 GATCAGGGGACAAATACTACAAGCAGAAGTACTCCAGGAACGACTGGAATGGAGAATCAG
 AGGAGTACAACAGCGCGCAAAGAGCTGGTGAAGTCAATCAGGCGATTTGGAGAGAGCT
 ATATTTCCGAGAAGACCAAGGGGAGATTTCTCAGCCTGGGGCGGCTATCAACGAGCACA
 AGAACGGCTCTGGGGGGAACAATCCTCACCAGGGTCTTAGACCTGGAGATTTCGAAGCG
 70 AAGGAGGAAACATTTATGACTGTTGCATTAAAGCCCAAGAGAACTCTCGCTATCCCTT
 GCTGTGGATTTCCCTTATGGCTATTTTGGGACTAGTAATTATAGTAGGACGCATAGCAG
 GCTATGGATTACGTGGACTCGCTGTTATAATAAGGATTTGTATTAGAGGCTTAAATTTGA
 TATTTGAAATAATCAGAAAAATGCTTGATTATATTGGAAGAGCTTTAAATCCTGGCACAT
 CTCATGTATCAATGCCTCAGTATGTTAGAAAAACAAGGGGGAACTGTGGGGTTTTTAT
 75 GAGGGGTTTTTATAAATGATTATAAGAGTAAAAAGAAAGTTGCTGATGCTCTCATAACCTT
 GTATAACCCAAAGGACTAGCTCATGTTGCTAGGCAACTAAACCGCAATAACCGCATTTGT

GACGCGAGTTCCCCATTGGTGACGCGTTAACTTCCTGTTTTACAGTATATAAGTGCTTG
 TATTCTGACAATTGGGCACTCAGATTCTGCGGTCTGAGTCCCTTCTCTGCTGGGCTGAAA
 AGGCCTTTGTAATAATAATAATTCTCTACTCAGTCCCTGTCTCTAGTTTGTCTGTCGAG
 5 ATCCTACAGAGCTCATGCCTTGGCGTAATCATGGTCATAGCTGTTTCTGTGTGAAATTG
 TTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGG
 TGCCTAATGAGTGAGCTAACTACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTC
 GGGAAACCTGTGCTGCCAGCTGCATTAAATGAATCGGCCAACGCGCGGGGAGAGCGGTTT
 GCGTATTGGGCGCTCTCCGCTTCCCTCGCTCACTGACTCGCTGCGCTCGGTCTGCTCGGCT
 10 GCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGA
 TAACGCAGGAAAGACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGC
 CGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCAGAAAAATCGACG
 CTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGG
 AAGCTCCCTCGTGCCTCTCTCTGTTCCGACCTGCCGCTTACCGGATACCTGTCCGCTT
 15 TCTCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCAGCTGTAGGTATCTCAGTTCCGT
 GTAGGTCGTTGCTCCAAGCTGGGCTGTGTGCACGAACCCCCGTTACGCCCCAGCGCTG
 CGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACT
 GGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTT
 CTTGAAGTGGTGGCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCT
 20 GCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAACAAACCAC
 CGCTGGTAGCGGTGGTTTTTTTGTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATC
 TCAAGAAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAACTCAG
 TTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTACCTAGATCCTTTTAAATTA
 AAAATGAAGTTTTAAATCAATCTAAAGTATATATAGTAAACTTGGTCTGACAGTTACCA
 25 ATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTTCATCCATAGTTGC
 CTGACTCCCCGTCGTGTAGATACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGC
 TGCAATGATACCGCGAGACCCACGCTCACC GGCTCCAGATTATCAGCAATAAACCAGCC
 AGCCGGAAGGGCCGAGCGCAGAAGTGGTCTGCAACTTTATCCGCTCCATCCAGTCTAT
 TAATTTGTTGCCGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGGCAACGTTGT
 30 TGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCTGTTGGTATGGCTTCATTCAGCTC
 CGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAAGCGGTAG
 CTCCTTCGGTCTCCGATCGTTGTGCAAGTAAGTTGGCCGAGTGTATCACTCATGGT
 TATGGCAGCACTGCATAATTCTCTTACTGTCTATGCCATCCGTAAGATGCTTTTCTGTGAC
 TGGTGAGTACTCAACCAAGTCATCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTG
 35 CCGGCGCTCAATACGGGATAATACCGCGCCACATAGCAGAAGTTTAAAGTGCTCATCAT
 TGGAAACAGTTCTTCGGGGCGAAAACCTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTC
 GATGTAACCCACTCGTGACCCCACTGATCTTCAGCATCTTTTACTTTCACCGAGCTTTC
 TGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAGGGAATAAGGGCGACACGGAA
 ATGTTGAATACTCATCTCTCTCTTTTCAATATTATTGAAGCATTATCAGGGTTATTG
 40 TCTCATGAGCGGATACATATTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCG
 CACATTTCCCCGAAAAGTGCCACCTAAATTGTAAGCGTTAATATTTGTTAAATTCGCG
 TTAATTTTTGTTAAATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCT
 TATAAATCAAAAGAAATAGACCGAGATAGGGTTGAGTGTGTTCCAGTTTGAACAAGAGT
 45 CCACTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAACCGTCTATCAGGGCGAT
 GGCCCACTACGTGAACCATCACCTTAATCAAGTTTTTGGGGTCGAGGTGCCGTAAGCA
 CTAATCGGAACCTTAAAGGGAGCCCGGATTTAGAGCTTGACGGGGAAAGCCAACCTGG
 CTTATCGAAATTAATACGACTCACTATAGGGAGACCGGC

SEQ ID NO 4 pSmart 2 MCS 5'cppt

50 AGATCTTGAATAATAAAATGTGTGTTTGTCCGAAATACGCGTTTTGAGATTTCTGTGCGC
 GACTAAATTCATGTCGCGCATAGTGGTGTATTCGCGGATAGAGATGGCGATATTGGAA
 AAATTGATATTGAAAATATGGCATATTGAAAATGTCGCCGATGTGAGTTTCTGTGTAAC
 55 TGATATCGCCATTTTCCAAAAGTGATTTTGGGCATACGCGATATCTGGCGATAGCGCT
 TATATCGTTTACGGGGGATGGCGATAGACGACTTTGGTGACTTGGGCGATTCTGTGTGTC
 GCAAATATCGCAGTTTCGATATAGGTGACAGACGATATGAGGCTATATCGCCGATAGAGG
 CGACATCAAGCTGGCAGATGGCCAATGCATATCGATCTATACATTGAATCAATATTGGCC
 ATTAGCCATATTATTCATTGGTTATATAGCATAAAATCAATATTGGCTATTGGCCATTGCA
 60 TACGTTGTATCCATATCGTAATATGTACATTTATATTGGCTCATGTCCAACATTACCGCC
 ATGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCA
 TAGCCCATATATGGAGTTCCGCGTTACATAACTACGGTAAATGGCCCGCTGGCTGACC
 GCCAACGACCCCCGCCCATTTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAAT
 AGGCACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGT
 65 ACATCAAGTGTATCATATGCCAAGTCCGCCCCCTATTGACGTCAATGACGGTAAATGGCC
 CGCCTGGCATATTGCCCAGTACATGACCTTACGGGACTTTCCCTACTTGGCAGTACATCTA
 CGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACACCAATGGGCGTGG
 ATAGCGGTTTGACTCACGGGGATTTCGAAGTCTCCACCCCATTTGACGTCAATGGGAGTTT
 GTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTGGCATCGCCCGCC
 70 CCGTTGACGCAAAATGGGCGGTAGGCGGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGT
 TTAGTGAACCGGCACATGATTCTGCGGTCTGAGTCCCTTCTCTGCTGGGCTGAAAAGG
 CCTTTGTAATAATAATAATTCTCTACTCAGTCCCTGTCTCAGTTTGTCTGTTGAGATC

CTACAGTTGGCGCCCGAACAGGGACCTGAGAGGGGCGCAGACCCCTACCTGTTGAACCTGG
 CTGATCGTAGGATCCCCGGGACAGCAGAGGAGAACTTACAGAAGTCTTCTGGAGGTGTTT
 CTGGCCAGAACACAGGAGGACAGGTAAGATTGGGAGACCCCTTGGACATTGGAGCAAGGCG
 5 CTCAAGAAGTTAGAGAAGGTGACGGTACAAGGGTCTCAGAAATTAAGTACTGGTAACTGT
 AATTGGGCGCTAAGTCTAGTAGACTTATTTTCATGATACCAACTTTGTAAGAGAAAGGAC
 TGGCAGCTGAGGGATGTCTTCCATTGCTGGAAGATGTAAGTCAAGACGCTGTGAGGACAA
 GAAAGAGAGGCTTTGAAAGAACATGGTGGGCAATTTCTGCTGTAAGATGGGCTCCAG
 ATTAATAATGTAGTAGATGGAAGGCATCATTCCAGCTCCTAAGAGCGAAATATGAAAAG
 AAGACTGCTAATAAAAGCAGTCTGAGCCCTCTGAAGAAATATCTCTAGAGTCGACGCTCT
 10 CATTAATTGTAACAAAGGGAGGAAAGTATGGGAGGACAGACCCATGGGAAGTATTAT
 CACTAATCAAGCACAAAGTAATACATGAGAACTTTTACTACAGCAAGCACAACTCCTCCAA
 AAAATTTTGTTTTACAAAATCCCTGGTGAACATGGTCGACTCTAGAACTAGTGGATCCC
 CCGGGCTGCGAGAGTGGGGAGGCACGATGGCCGCTTTGGTCGAGGCGGATCCGGCCATTA
 GCCATATTATTCATTGGTTATATAGCATAAATCAATATTGGCTATTGGCCATTGCATACG
 15 TTGTATCCATATCATAATATGTACATTTATATTGGCTCATGTCCAACATTACCGCCATGT
 TGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGC
 CCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCC
 AACGACCCCCGCCATTGACGTCATAATGACGTATGTTCCCATAGTAACGCCAATAGGG
 ACTTTCCATTGACGTCAATGGGTGGAGTATTACGGTAAACTGCCCACTTGGCAGTACAT
 20 CAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCC
 TGGCATTATGCCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTA
 TTAGTCATCGCTATTACCATGGTGTATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAG
 CGGTTTGACTCAGCGGGATTTCCAGTCTCCACCCCACTTACGCTCAATGGGAGTTTGT
 TGGCACCAAAATCAACGGGACTTTCCAAAATGTCTGAACAACTCCGCCCACTTGACGCCAA
 25 ATGGGCGGTAGGCATGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCGT
 CAGATCGCCTGGAGACGCCATCCACGCTGTTTGGACCTCCATAGAAGACACCGGGACCGA
 TCCAGCCTCCGCGGCCCAAGCTTTGGTACCTTGTAGCTTGGCGCGCTTCTCGAGTT
 GGGCCCGGACCTTACTAGGTTGCTGTGGAAGGGTGTGGTGCAGTAGTAGTTAATGATGAA
 GGAAAGGGAATAATTGCTGTACCATTAACCAGGACTAAGTACTAATAAAACCAATTGA
 30 GTATTGTTGCAAGGAAGCAAGACCAACTACCATTTGTCAGCTGTGTTCTGACCTCAATA
 TTTGTTATAAGGTTTGATATGAATCCCAGGGGGAATCTCAACCCCTATTACCAACAGTC
 AGAAAAATCTAAGTGTGAGGAGAACACAATGTTTCAACCTTATTGTTATAATAATGACAG
 TAAGAACAGCATGGCAGAATCGAAGGAAGCAAGAGACCAAGAATGAACCTGAAAGAAGAA
 TCTAAAGAAGAAAAAGAAAGAAATGACTGGTGGAAAATAGGTATGTTTCTGTTATGCTTA
 35 GCAGGAACCTACTCGAGGAATACTTTGGTGGTATGAAGGACTCCACAGCAACATATATA
 GGGTTGGTGGCGATAGGGGGAAGATTAAACGGATCTGGCCAAATCAATGCTATAGAATGC
 TGGGGTTCCCTTCCCGGGGTGTAGACCATTTCAAAATTACTTCAGTTATGAGACCAATAGA
 AGGTGACCAAGTGGTGCAGGTCCTCCGGCAGTCTTACCTGAAGAAAAATTCATCACA
 AACATGCATCGCGAGAGACACCTGGGACCAAGGCCAACACAACATACACCTAGCAGGCG
 40 TGACCGGTGGATCAGGGGACAATACTACAAGCAGAAGTACTCCAGGAACGACTGGAATG
 GAGAATCAGAGGAGTACAACAGGCGGCCAAAGAGCTGGGTGAAGTCAATCGAGGCATTTG
 GAGAGAGCTATATTTCCGAGAAGACCAAGGGGAGATTTCAGCCTGGGGCGGCTATCA
 ACGAGCACAAAGACGGCTCTGGGGGGAACAATCCTCACCAAGGGTCTTAGACCTGGAGA
 TTCGAAGCGAAGGAGGAAACATTTATGACTGTTGCATTAAAGCCCAAGAGGAACCTCTCG
 45 CTATCCCTTGCTGTGGATTTCCTTATGGCTATTTTGGGACTAGTAATTATAGTAGGAC
 GCATAGCAGGCTATGGATTACGTGGACTCGCTGTTATAATAAGGATTTGTATTAGAGGCT
 TAAATTTGATATTTGAAATAATCAGAAAAATGCTTGATTATATTAGCTTCGATCACTAGT
 GAATTCGCGGCGCTTAATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTAT
 50 TCTTAACCTATGTTGCTCCTTTTACGCTATGTGGATACGCTGCTTAAATGCCTTTGTATCA
 TGCTATTGCTTCCCGTATGGCTTTTCATTTTCTCCTCTGTATAAATCCTGGTTGCTGTC
 TCTTTATGAGGAGTTGTGGCCGTTGTGAGGCAACGTGGCGTGGTGTGCACTGTGTTTGC
 TGACGCAACCCCACTGTTGGGGCATTGCCACCACCTGTCAGCTCCTTTCCGGGACTTT
 CGCTTTCCCTCCCTATTGCCACGGCGGAACATCATCGCCGCTGCTTGGCCGCTGCTG
 55 GACAGGGGCTCGGCTGTGGGCACTGACAATTCGCTGGTGTGTGCGGGGAAGCTGACGTC
 CTTTCCATGGCTGCTCGCTGTGTTGCCACCTGGATTCTGCGCGGGACGTCCTTCTGCTA
 CGTCCCTTCGGCCCTCAATCCAGCGGACCTTCTTCCCGCGGCTGCTGCCGGCTCTGCG
 GCCTCTTCCGCGCTCTTCGCTTCGCCCTCAGACGAGTCGGATCTCCCTTTGGGCGCCTC
 CCCGCTGATCGATCTCGACATCGAATTCGCCGCGCGCTCGACAGCTATCGATACCGT
 60 CGAATTGGAAGAGCTTTAAATCCTGGCACATCTCATGTATCAATGCCTCAGTATGTTAG
 AAAACAAGGGGGAACTGTGGGTTTTATGAGGGTTTTATACAATTGGGCACCTCAGA
 TTCTGCGGTCTGAGTCCCTTCTCTGCTGGGCTGAAAAGGCCTTGTAAATAATATAATTC
 TCTACTCAGTCCCTGTCTCTAGTTTGTCTGTTTCGAGATCCTACAGAGCTCATGCCTTGGC
 GTAATCATGGTTCATAGCTGTTTCTGTGTGAAATTTGTTATCCGCTCACAATTCACACAA
 65 CATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCTTAATGAGTGAGCTAACTCAC
 ATTAATTGCGTTGCGCTCACTGCCGCTTTCCAGTCGGGAAACCTGTGTCGCCAGCTGCA
 TTAATGAATCGGCAACCGCGGGGAGAGGCGGTTTGCCTATTGGGCGCTCTTCCGCTTC
 CTCGCTCACTGACTCGCTGCGCTCGGTCGTTGCGCTGCGGCGAGCGGTATCAGCTCACTC
 AAAGGCGGTAAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGC
 AAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCTTGGTGGCGTTTTTCCATAG
 70 GCTCCGCCCCCTGACGAGCATCACAATAATCGACGCTCAAGTCAGAGGTGGCGAACC
 GACAGGACTATAAGATACAGGCGTTTCCCTCGGAAGCTCCCTCGTGGCTCTCTGT
 TCCGACCTGCGCTTACCGGATACCTGTCCGCTTCTCCCTTCGGGAAGCGTGGCGCT
 TTCTCATAGCTCAGCTGTAGGTATCTCAGTTCGGTGTAGGTGCTTCGCTCCAAGCTGGG
 75 CTGTGTGCACGAACCCCCGTTACGCCGACCGCTGCGCTTATCCGGTAACATATCGTCT
 TGAGTCCAACCCGTAAGACACGACTTATCGCCACTGGCAGACGCACTGGTAACAGGAT

TAGCAGAGCGAGGTATGTAGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGG
 CTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAA
 AAGAGTTGGTAGCTCTTGATCCGGCAAACAACCACCGCTGGTAGCGGTGGTTTTTTTGT
 5 TTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCCTTGATCTTTTC
 TACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATT
 ATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTA
 AAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTAT
 CTCAGCGATCTGTCTATTTTCGTTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAAC
 10 TACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACG
 CTCACCGGCTCCAGATTTATCAGCAATAAACCCAGCCAGCCGGAAGGGCCGAGCGCAGAAG
 TGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGT
 AAGTAGTTCGCCAGTTAATAGTTTTCGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGT
 GTCACGCTCGTCGTTTGGTATGGCTTCATTTCAGCTCCGGTTCCCAACGATCAAGGGCAGT
 TACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGT
 15 CAGAAGTAAGTTGCCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCT
 TACTGTCTATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATT
 CTGAGAAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATAC
 CGCGCCACATAGCAGAACTTTAAAAGTGCTCATCTATTGGAACGTTCTTCGGGGCGAAA
 ACTCTCAAGGATCTTACCCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAA
 20 CTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCA
 AAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAATGTTGAATACTCATACTCTTCCT
 TTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGA
 ATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACC
 TAAATTGTAAGCGTTAATATTTTGTAAAAATTCGCGTTAAATTTTGTAAATCAGCTCA
 25 TTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAAATAGACCGAG
 ATAGGGTTGAGTGTGTTCCAGTTTGGAAACAAGAGTCCACTATTAAAGAACGTGGACTCC
 AACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCCACTACGTGAACCATCACCC
 TAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCTTAAAGGGAGC
 CCCCATTATAGAGCTTGACGGGGAAGCCAACCTGGCTTATCGAAATTAATACGACTCAC
 30 TATAGGGAGACCGGC

CLAIMS

1. Use of a viral vector system to transduce a target adipose tissue site.
- 5 2. The use of a viral vector system according to claim 1, wherein the viral vector is pseudotyped with a nucleotide sequence that encodes at least a part of an env protein.
3. The use of a viral vector system according to claim 1 or claim 2, wherein the
10 env protein is a Rabies G protein or a mutant, variant, homologue or fragment thereof.
4. The use of a viral vector system according to claim 1 or claim 2, wherein the
env protein is a VSV-G protein, coccal virus glycoprotein, or a chandipura virus
glycoprotein or a mutant, variant, homologue or fragment thereof.
- 15 5. The use according to any one of the preceding claims, wherein the viral vector system is derivable from the group selected from a retrovirus, a pox virus, a herpes virus and a baculovirus.
6. The use according to any one of the preceding claims, wherein the viral vector
20 system is derivable from the group selected from an adenovirus and an adeno-associated virus.
7. The use according to claim 5, wherein the retrovirus system is derivable from
a lentivirus.
- 25 8. The use according to claim 7, wherein the lentiviral vector system is derivable from ELAV or HIV.
9. Use of a viral vector system to transduce a target adipose tissue site, wherein
30 the viral vector is pseudotyped with a nucleotide sequence that encodes at least a part of an env protein.

10. The use of a viral vector system according to claim 9, wherein, the env protein is a Rabies G protein or a mutant, variant, homologue or fragment thereof.

11. The use of a viral vector system according to claim 10, wherein the env
5 protein is a VSV-G protein, coccal virus glycoprotein, or a chandipura virus glycoprotein or a mutant, variant, homologue or fragment thereof.

12. The use according to claims 9 to 11, wherein the viral vector system is derivable from the group selected from a retrovirus, a pox virus, a herpes virus and a baculovirus.

10

13. The use according to claims 9 to 11, wherein the viral vector system is derivable from the group selected from an adenovirus and an adeno-associated virus.

14. The use according to claim 12, wherein the retrovirus system is derivable from
15 lentivirus.

15. The use according to claim 14, wherein the lentiviral vector system is derivable from EIAV or HIV.

20 16. Use of a viral vector system to transduce/infect a target adipose tissue site, wherein the viral vector is derivable from the group selected from a retrovirus, a pox virus, a herpes virus and a baculovirus.

17. Use of a viral vector system to transduce/infect a target adipose tissue site,
25 wherein the viral vector is derivable from the group selected from an adenovirus, an adeno-associated virus.

18. The use according to claim 16, wherein the retrovirus system is derivable from lentivirus.

30

19. The use according to claim 18, wherein the lentiviral vector system is derivable from EIAV or HIV.

20. The use of a viral vector system as defined in any one of claims 16 to 19, wherein the viral vector is pseudotyped with a nucleotide sequence that encodes at least a part of an env protein.

5 21. The use of a viral vector system according to claim 20, wherein the env protein is a Rabies G protein or a mutant, variant, homologue or fragment thereof.

22. The use of a viral vector system according to claim 20, wherein the env protein is a VSV-G protein, coxal virus glycoprotein, or a chandipura virus glycoprotein or a mutant, variant, homologue or fragment thereof.

10 23. The use according to any one of the preceding claims, wherein the viral vector system comprises at least one NOI.

24. The use according to any one of the preceding claims, wherein the NOI is a selection gene, a marker gene, a therapeutic gene, an antisense sequence or a cDNA library.

25. The use according to any one of the preceding claims, wherein the NOI is capable of blocking or inhibiting the expression of a gene in a target cell.

20 26. The use according to any one of the preceding claims, wherein at least part of the NOI is capable of integrating in the genome of a target cell.

27. The use according to any one of the preceding claims, wherein the NOI has a therapeutic effect or codes for a protein that has a therapeutic effect.

28. The use according to any one of the preceding claims, wherein the NOI is capable of encoding a protein of interest ("POI").

30 29. The use of a viral vector system as defined in any one of the preceding claims, in the manufacture of a pharmaceutical composition to treat and/or prevent a disease in a subject.

30. A method of treating and/or preventing a disease in a subject in need of same, said method comprising the step of using a viral vector system as defined in any one of claims 1 to 28 to transduce/infect a target adipose tissue site.

5 31. A method according to claim 30, to treat and/or prevent a disease which is associated with a derangement in the metabolism of adipose tissue.

32. A method according to claim 30 or 31, to treat and/or prevent obesity and/or diabetes.

10

33. A method for analysing the effect of a POI in a target adipose tissue site comprising the step of using a viral vector system as defined in claim 28.

15 34. A method for analysing the function of a gene, or a protein encoded by a gene, in a target cell, which method comprises the step of inhibiting or blocking the expression of the gene using a viral vector system as defined in claim 25.

35. A target adipose tissue cell transduced/infected with a viral vector system as defined in any one of claims 1 to 28.

20

36. The use of a target adipose tissue cell according to claim 35 in the manufacture of a medicament for use in the prevention and/or treatment of a condition associated with adipose tissue metabolism.

25 37. A method for treating and/or preventing a disease in a subject in need of same, said method comprising the step of transplanting a target adipose tissue cell according to claim 36 into said subject.

30

ABSTRACT**VECTOR SYSTEM**

5

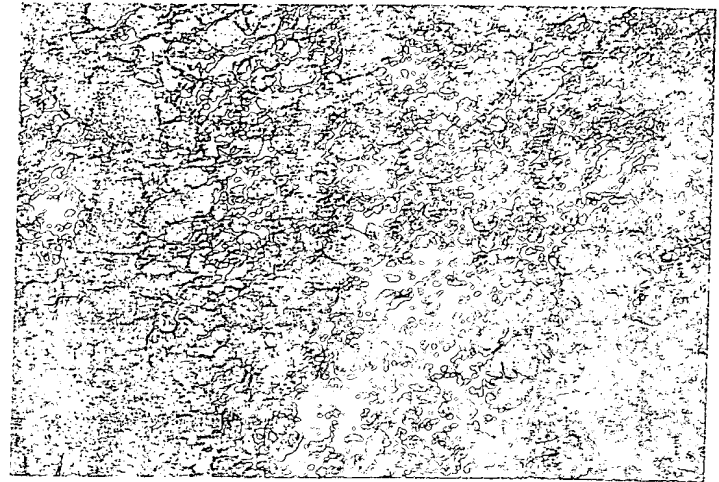
The invention provides the use of a viral vector system to transduce/infect a target adipose tissue site. The invention also provides a method of treating and/or preventing a disease in a subject and use of a viral vector system in the manufacture of a pharmaceutical composition to transduce/infect a target adipose tissue site
10 wherein the disease is associated with a derangement in the metabolism of adipose tissue.

15

4/14

β -galactosidase expression in the mammary fat pad tissue of nude mice, transduced with ElAV pONY 8Z 5' pos del CTS vector, 14 days post injection.

Section 1



4 x magnification

10x magnification

Section 2



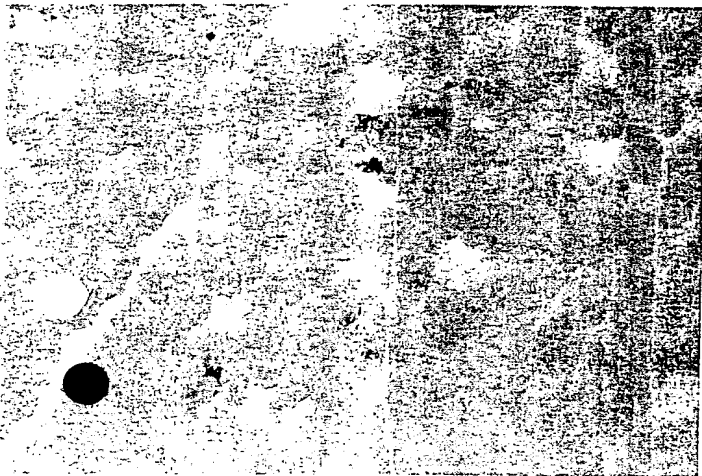
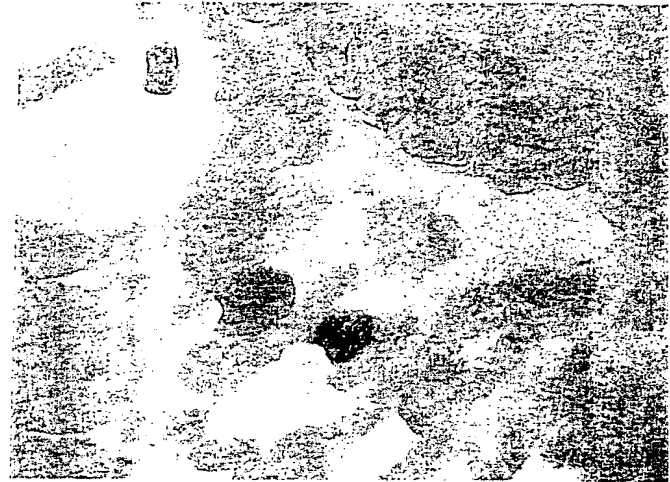
Figure 1

2/14

β -galactosidase expression in rat gastrocnemius muscle. 14 days after the injection of VSV-G pseudotyped EIAV pONY 8Z 5' pos del CTS vector.



10x mag



10x mag

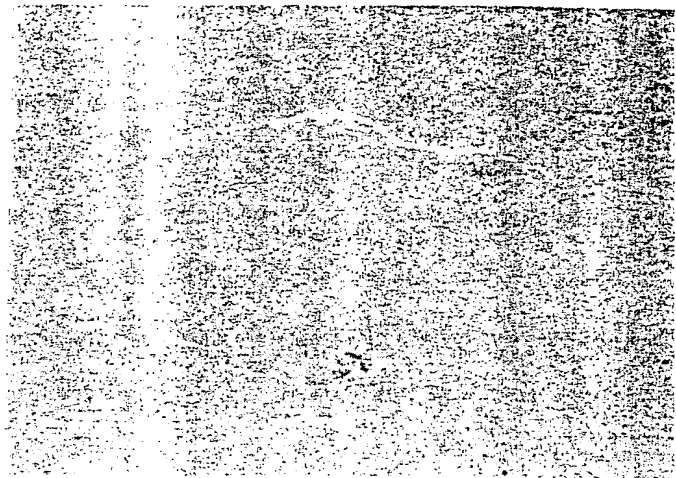
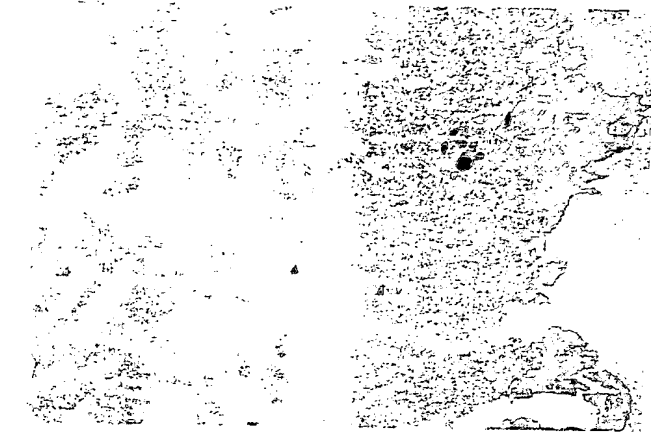


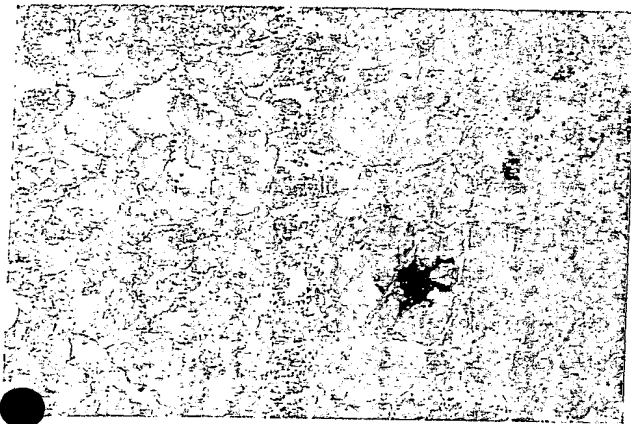
Figure 2

3/14

β -galactosidase expression in mouse
gastrocnemius muscle. 14 days after the injection
of VSV-G pseudotyped ElAV pONY 8Z 5' pos del
CTS vector.



4x mag



10x mag

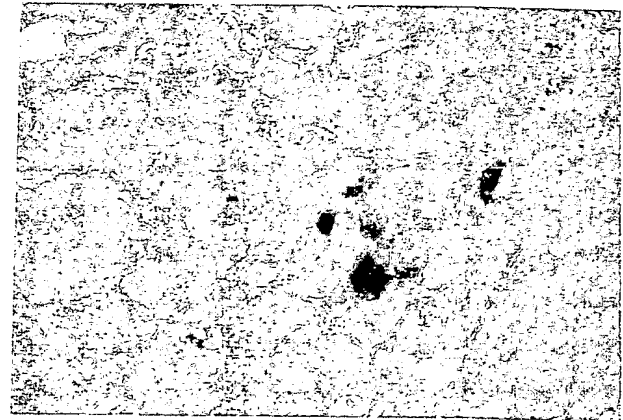
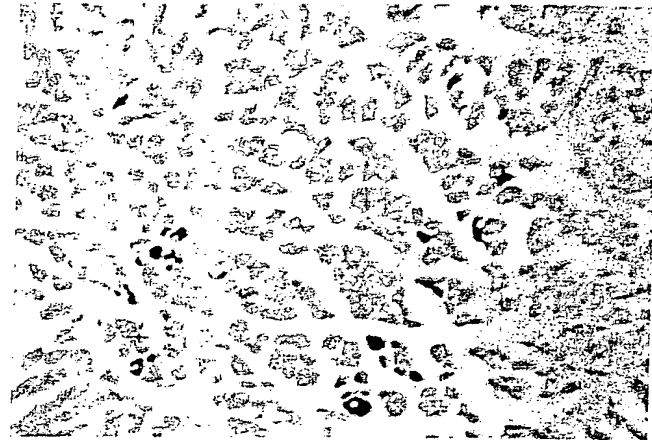


Figure 3

4/14

β -galactosidase expression in mouse
gastrocnemius muscle. 28 days after the injection
of VSV-G pseudotyped EIAV pONY 8Z 5' pos del
CTS vector.

4x



10x

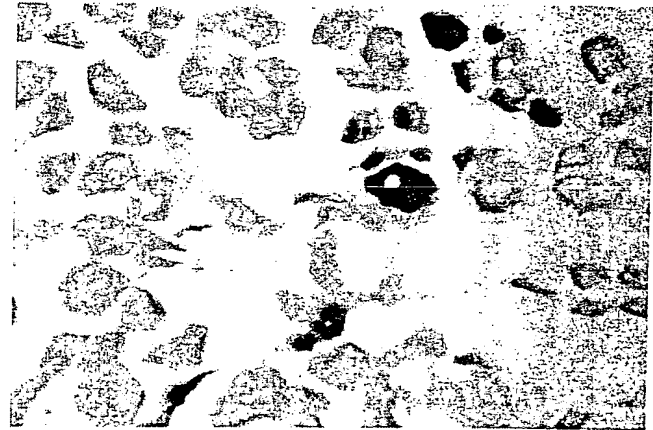


Figure 4

Staining could be found in both of the two animals examined.

5/14

β -galactosidase expression in rat gastrocnemius muscle. 28 days after the injection of VSV-G pseudotyped EIAV pONY 8Z 5' pos del CTS vector.

4x



10x



Figure 5

Staining could only be found in one of the two animals examined.

6/14
 β -galactosidase expression in mouse
gastrocnemius muscle. 84 days after the injection
of VSV-G pseudotyped EIAV pONY 8Z 5' pos del
CTS vector.

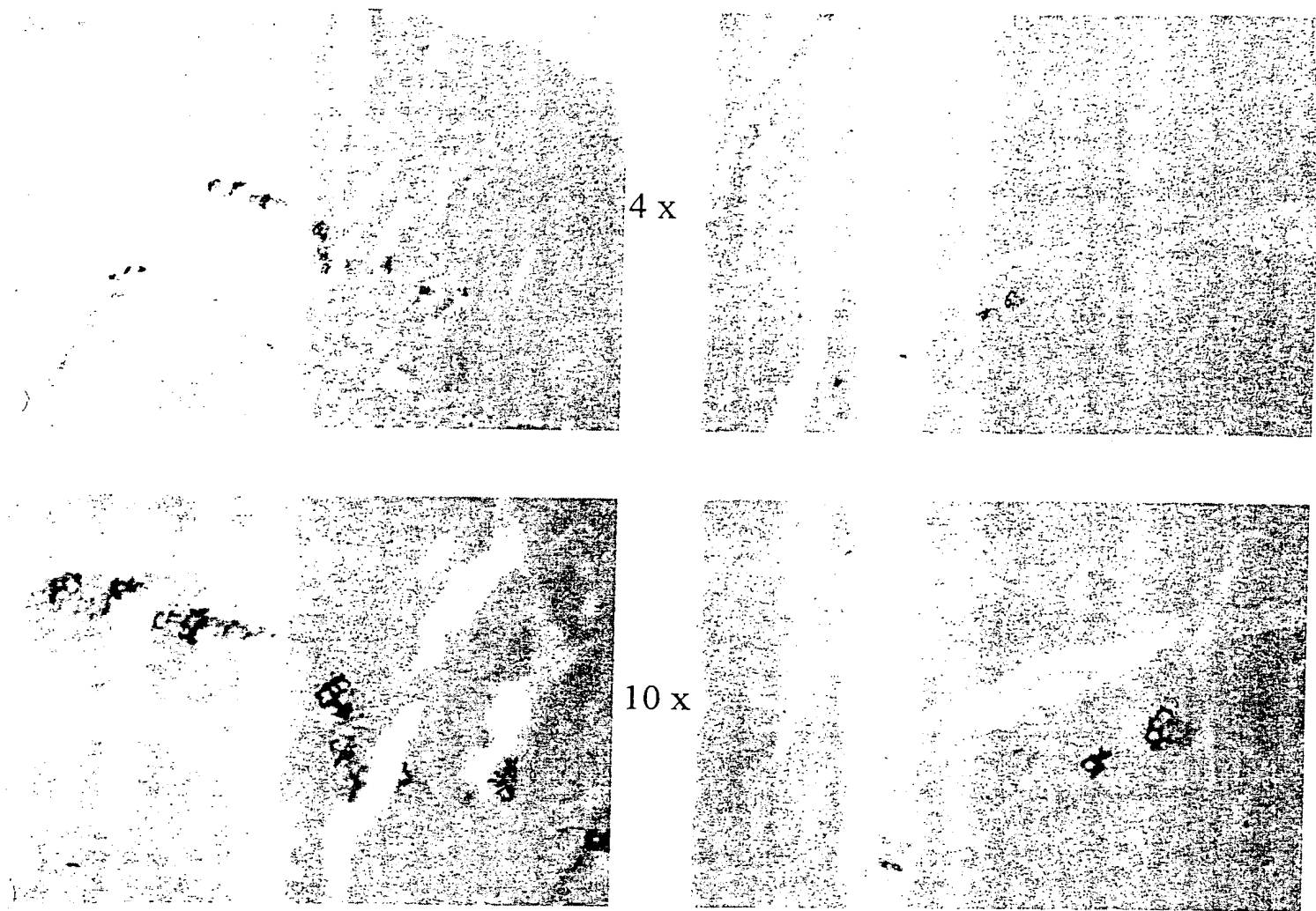


Figure 6

Staining could be found in both of the two animals examined.

7/14

β -galactosidase expression in rat gastrocnemius muscle. 84 days after the injection of VSV-G pseudotyped EIAV pONY 8Z 5' pos del CTS vector.

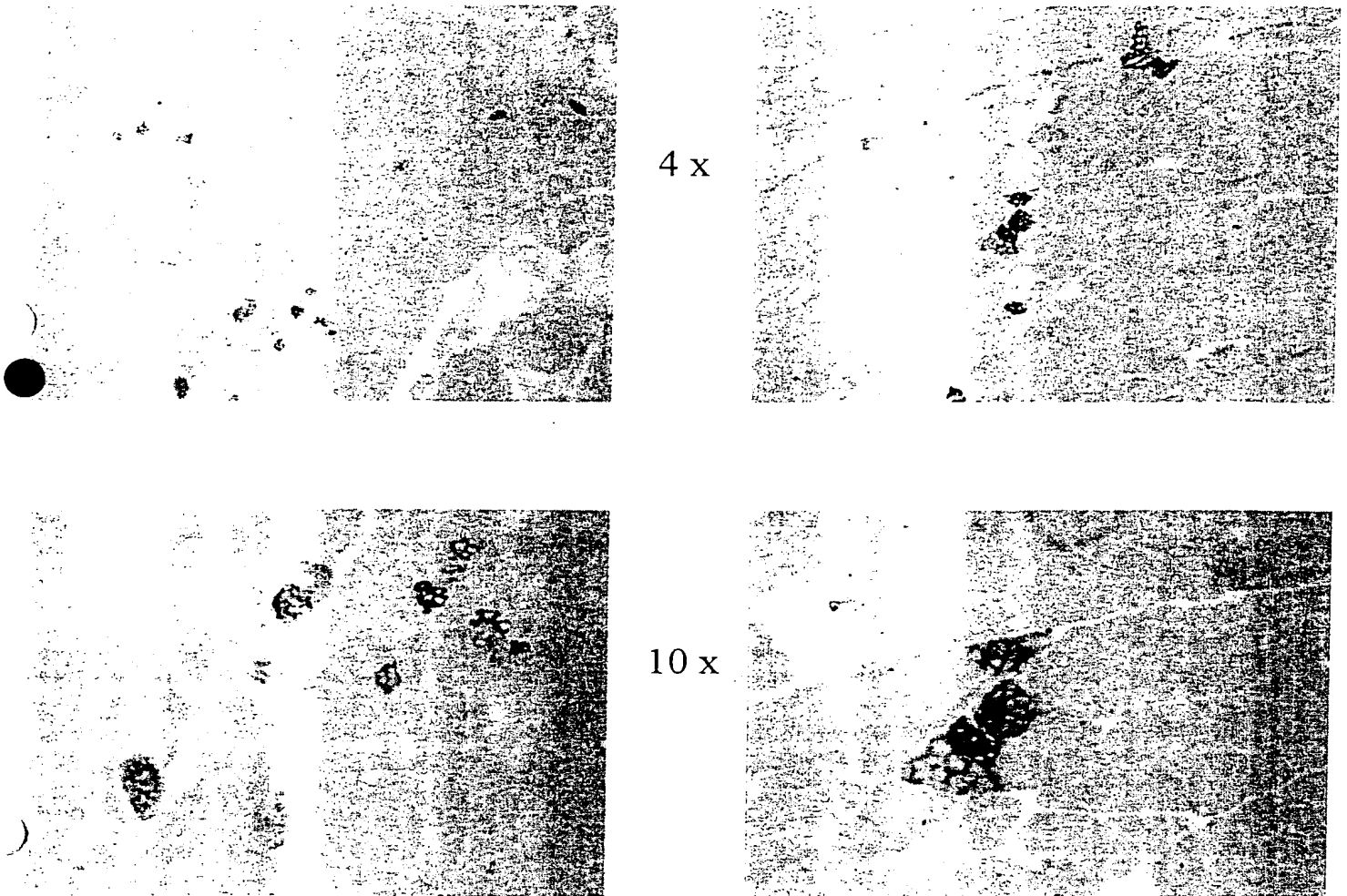


Figure 7

Staining could be found in both of the two animals examined.

8/14

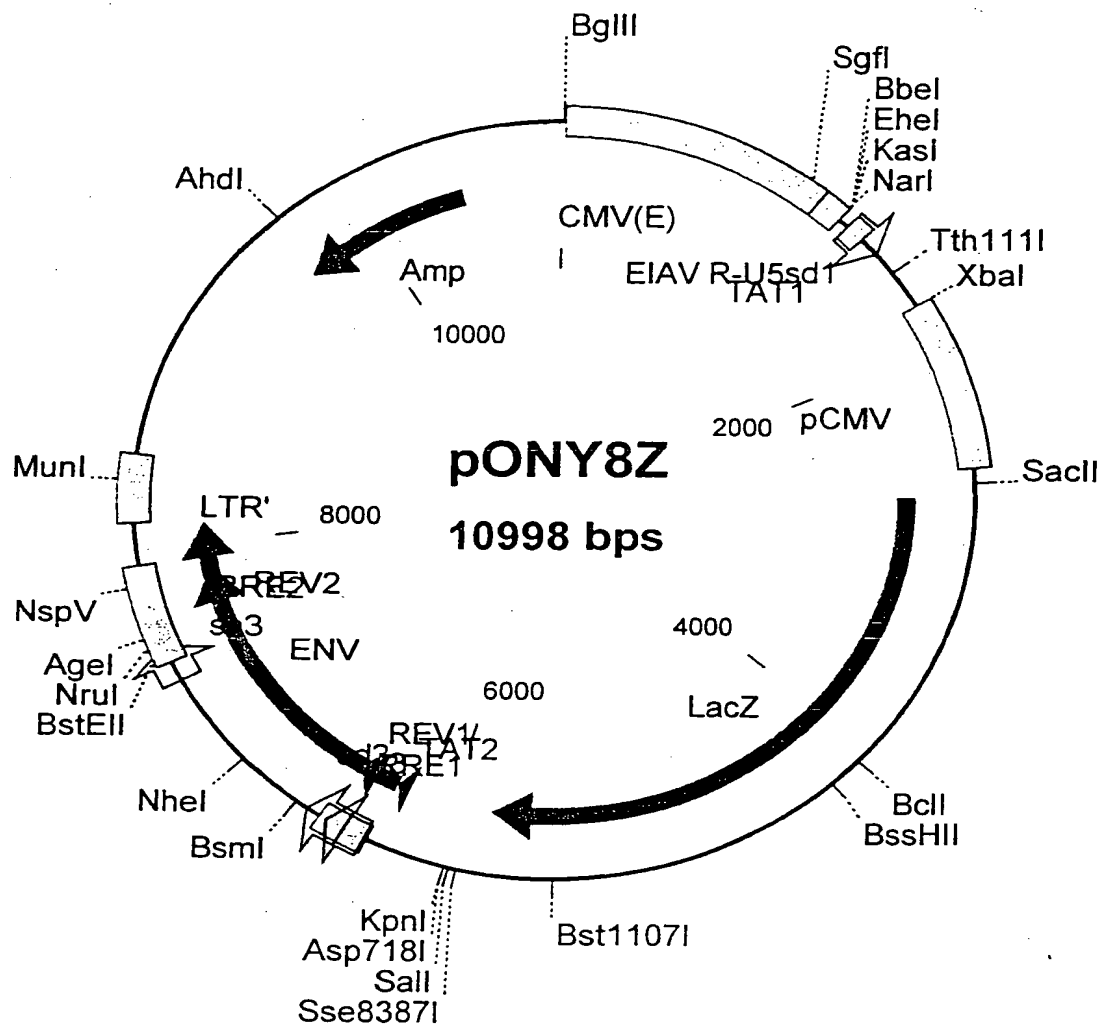


Figure 8

9/14

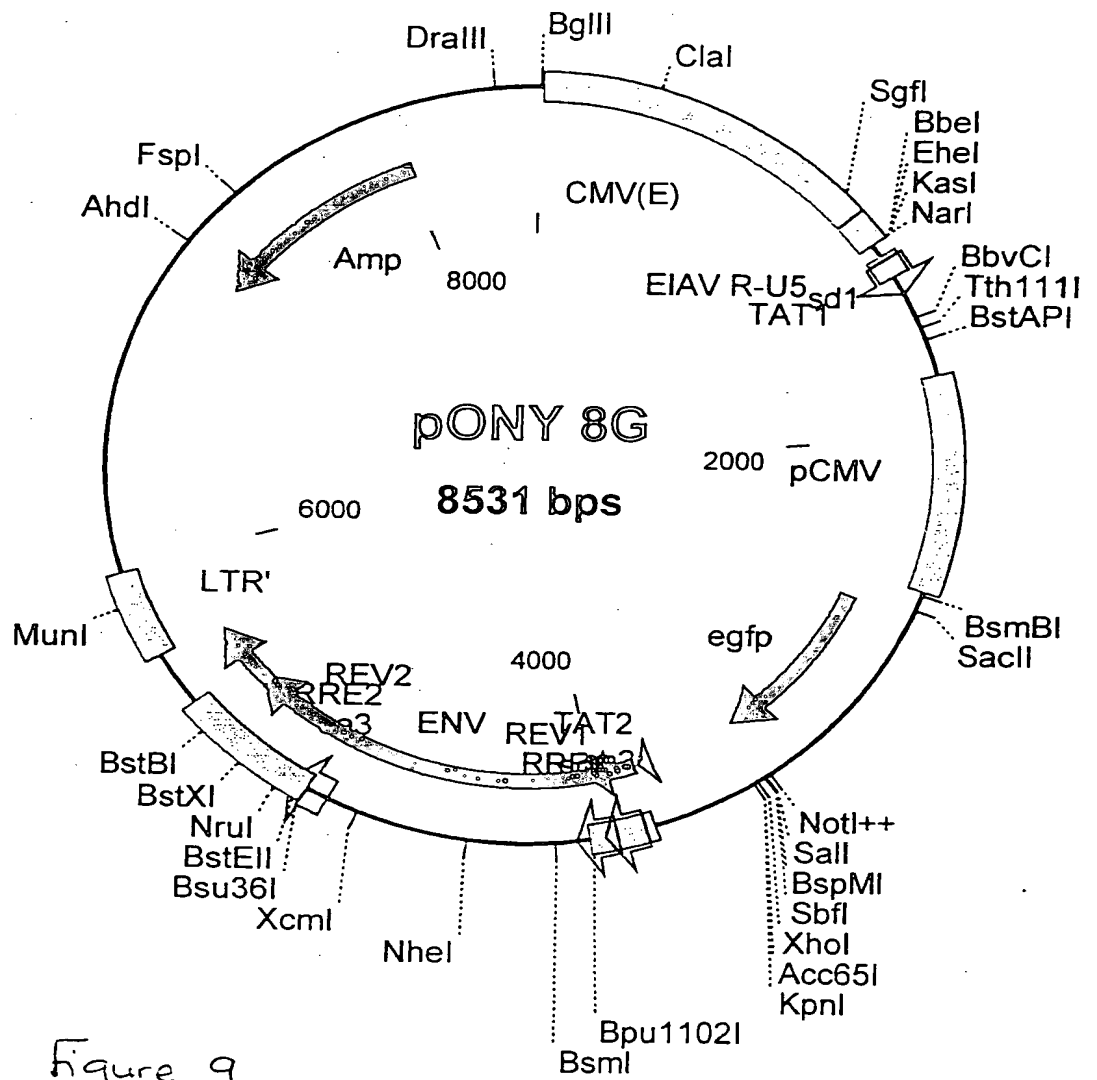


Figure 9

10/14

pONY8Z 5'POS delCTS

11019 bps

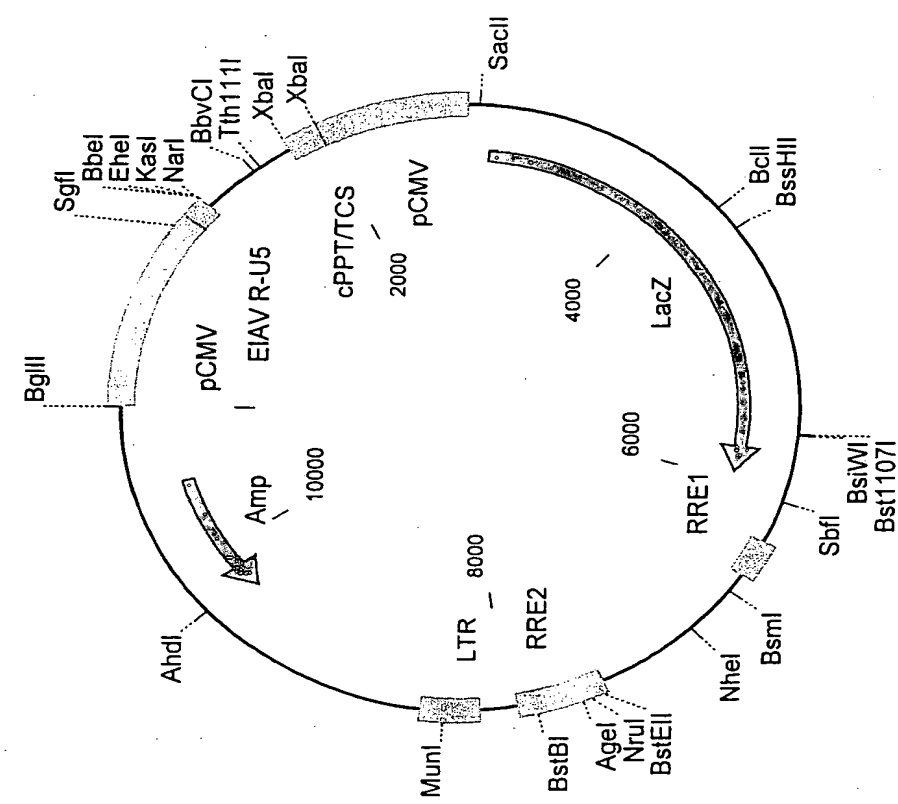


Figure 10

11/14

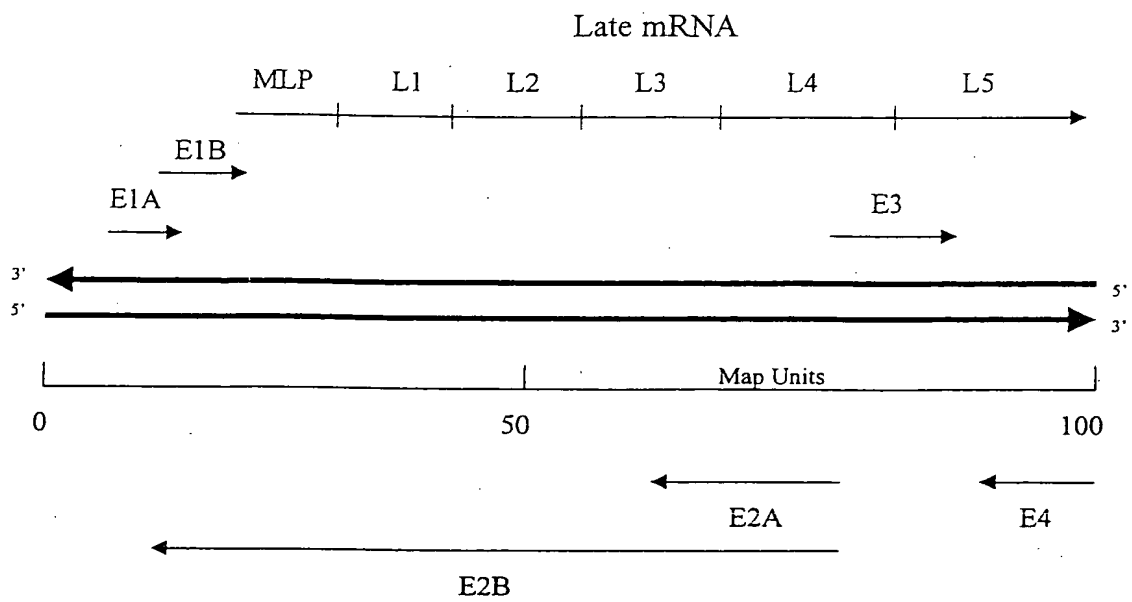


Figure 11

Features of pSmart2 MCS 5'cppt.

Start	End	feature
6	1150	human CMV early promoter
1151	1270	EIAV R-U5
1785	1962	cPPT./CTS
1963	2759	human CMV early promoter
2841	2885	multiple cloning site
4090	4688	WPRE1
4818	4965	EIAV self inactivating LTR sequences
7011	6151	ampicillin resistance

Figure 12

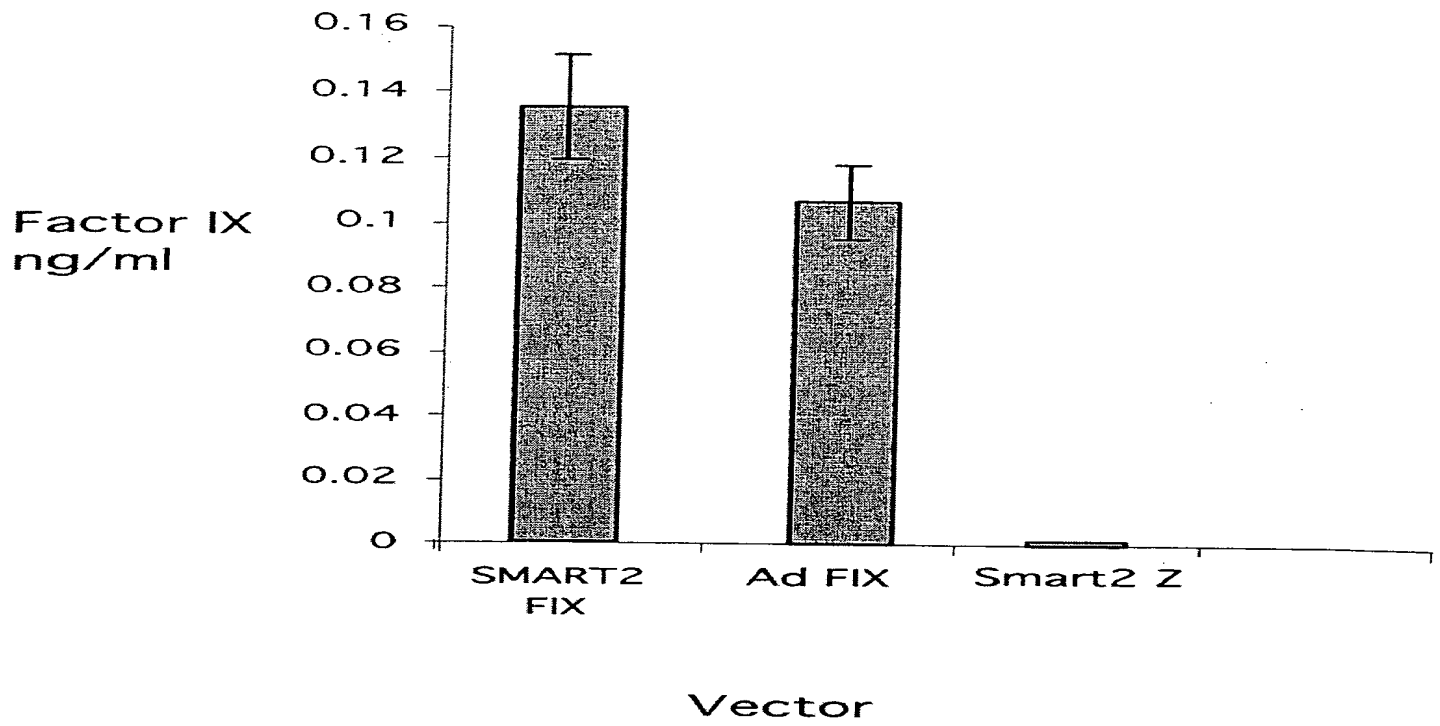


Figure 13

14/14

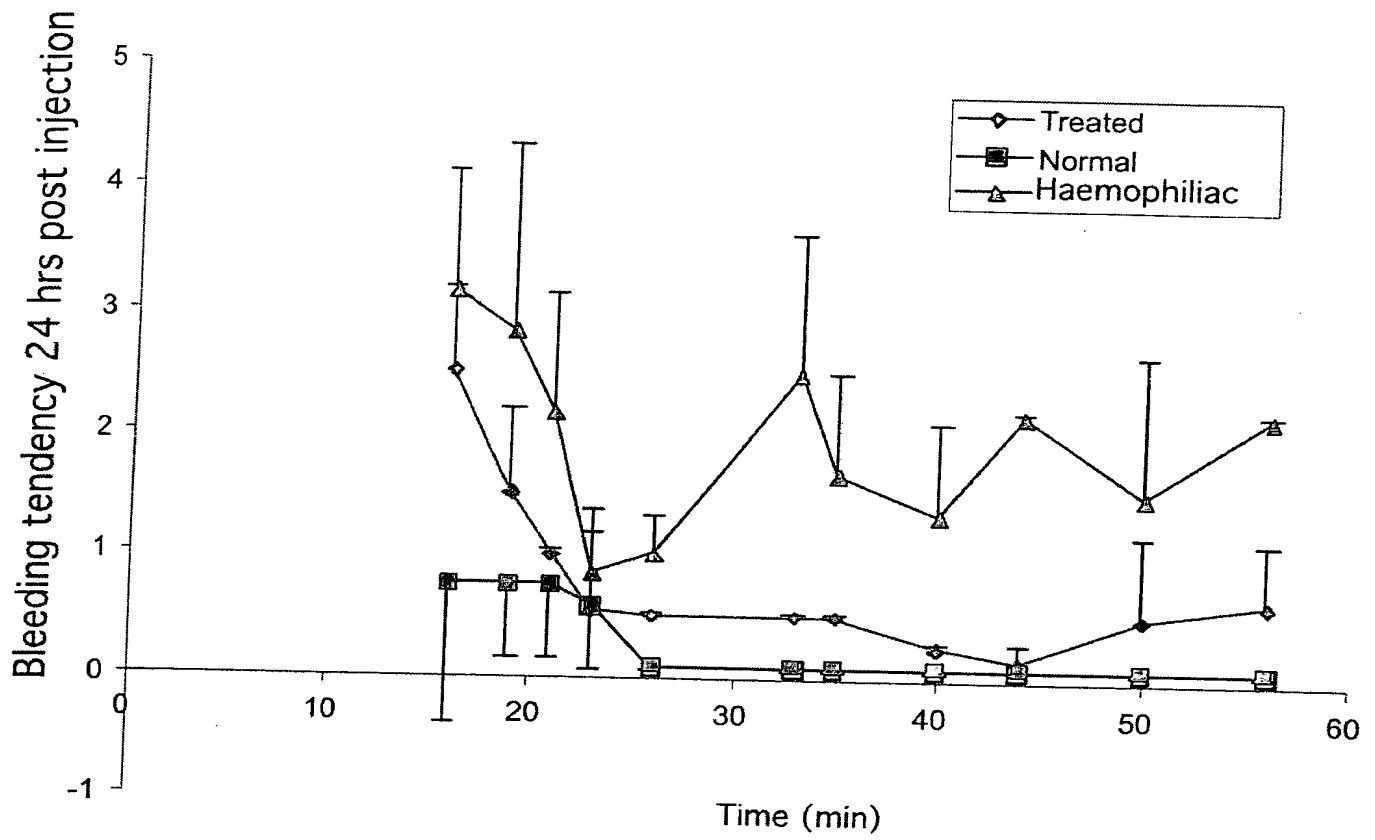


Figure 14